

## Research Article

# Comparison of Phytochemical Profile, Mineral Content, and *In Vitro* Antioxidant Activities of *Corchorus capsularis* and *Corchorus olitorius* Leaf Extracts from Different Populations

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Received 27 November 2019; Accepted 20 February 2020; Published 24 March 2020

Academic Editor: Flora V. Romeo

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In addition to fiber, *Corchorus* (jute) leaf is also rich in secondary metabolites and is used in folk medicine in jute-producing communities. It has been reported that jute halts progression of and helps manage different chronic diseases like tumors, obesity, diabetes, and cardiovascular diseases. Various phytochemical, mineral, and antioxidant potency properties of 30 genotypes belonging to *Corchorus capsularis* and *Corchorus olitorius* were evaluated in the current study. The results demonstrate that the range of total flavonoids and polyphenols was 3.04 to 13.66 mg rutin equivalent (RE)/g and 5.12 to 7.78 mg gallic acid equivalent (GAE)/g DW, respectively. Total tannin and saponin content in both species was 13.08 to 26.95 mg tannic acid (TAE)/g and 34.45 to 114.59 mg tea saponin (TSE)/g DW, respectively, when analyzed for the first time. Moreover, this study sincerely establishes that jute leaf is a great source of mineral elements (magnesium, zinc, and selenium) and could also be a good energy source. The antioxidant properties of samples were examined with three unique strategies, including DPPH, FRAP, and ABTS values of leaf extract ranging from 206.42 to 351.77  $\mu\text{g/ml}$  ( $\text{IC}_{50}$ ), 16.69 to 94.69 mmol Fe (II)/g DW, and 50.27 to 149.90 mmol trolox equivalent (TE)/g DW, respectively. A principle component analysis (PCA) explained 58.52% of the variance, while a hierarchical cluster analysis (HCA) was performed to construct five distinct groups based on their secondary compound metabolites content and antioxidant activities. Therefore, this study facilitates selection of the high genotypes that might be used as new materials for developing industrial and medicinal uses in addition to sorting genotypes for future genetic engineering purposes in order to enhance a particular bioactive compound and its natural antioxidants that are beneficial for human health.

## 1. Introduction

Jute (*Corchorus* sp.) is an annual flowering plant in the Tiliaceae family and the most significant source of natural fiber, covering about 80% of global bast fiber production [1]. Besides, young shoots and leaves are consumed as vegetable and food ingredients and have long been used as medicinal folk remedies in Eastern Asia and Africa. Health-flourishing effects of plant-derived secondary metabolites in human health, including antioxidative, anticarcinogenic, antibiotic, and pharmacological effects, are well documented [2]. *C. capsularis* and *C. olitorius* are commercially cultivated and these two species are major source of fiber from jute. Leaves of *C. olitorius*

possess an abundance of antioxidants compounds associated with various biological properties, which include diuretic, analgesic, antipyretic, and antimicrobial activities, antitumor [3] and phenolic antioxidative compounds [4], hypoglycemic [5], antiobesity [6], and gastroprotective [7]. Moreover, *C. capsularis* leaves are used in ayurvedic for ascites, piles, cystitis, dysuria, fever, and gonorrhoea [8]. Thus, from the ancient time jute leaves have been used in traditional medicine without being used as an actual curative source. Different experiments have revealed that plant parts, such as leaves, stems, roots, barks, and seeds, of *C. capsularis* and *C. olitorius* contain polysaccharides, flavonoids, phenolics, cardiac glycosides, sterols, fatty acids, triterpenoids, and ionones [9].

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 [10], which are related to some chronic diseases, like cancer, inflammation, cardiovascular diseases, and others [11]. Antioxidants may be defined as complex determined compounds that function as defensive shields against several diseases [12]. As antioxidants perform free radical chain reaction terminator through electron pairing with active biological macromolecules, they might be used to prevent aging-associated diseases and other health problems. Therefore, there is rising interest in the research of plant bioactive molecules and phenolic compounds due to the getting awareness for human health in addition to extensive applications in the food, cosmetic, herbal, and pharmaceutical industries as a natural antioxidant [13]. Plant bioactive molecules can replace synthetic ones, intervene with the oxidative stress-induced process, and, therefore, prevent alteration in lipids, proteins, and DNA [14].

Concentrations of these bioactive compounds are strongly dependent on genotypes [15]. The nutritional values and curative potential of the jute leaves have prompted investigations concerning chemical content and antioxidant potency of jute leaves in order to find promising new sources of natural antioxidants. Research with newly isolated compounds has shown that *C. olitorius* leaves are plentiful in phenolics and flavonoids in addition to possessing a high level of antioxidant and antimicrobial activities [16]. Flavonoids form an important group of natural low molecular weight phenolic compounds that are extensively distributed throughout in the plant kingdom and exhibit diverse biological characteristics, including those associated with antioxidative, antiviral, anti-inflammatory, and anticarcinogenic activities [17]. Because they are excellent electron donors and possess distinctive beneficial biological properties, polyphenolic compounds can directly contribute to antioxidant actions within a cell and have come to be attractive natural ingredients in the pharmaceutical and cosmetic industries [18, 19]. Because of their therapeutic properties, certain tannins have been shown to inhibit human immunodeficiency virus (HIV) replication [20], and tannins may be used in the treatment of different diseases to boost human health. Nevertheless, research about the phytochemical constituents of jute genotypes ought to assist in developing an extended understanding and perception of the nutraceutical and medicinal value of jute, which in turn could ameliorate its consumption. Furthermore, it is also necessary to signify the genotypes from a chemical point of view in order to allow them to fit into industrial demands with greater contents of fitness-promoting bioactive compounds.

Plants provide minerals, vitamins, and positive hormone precursors in addition to protein and energy to the human body [21]. Based on nutritional requirements, various minerals are categorized as macronutrients or micronutrients. At the same time, both major and trace elements play a significant role in the build-up and restorative development in health and sickness of a physical body. Some

studies have reported that mineral and trace elements such as Mg, Zn, and Se are considered as antioxidant elements. In terms of contemporary pharmacological ideas, correlation between elemental contents of edible plant parts and their healing capacity is not yet understood. Thus, quantitative estimations of various elements' concentrations are crucial for figuring out the effectiveness and relationship of the plant with secondary metabolites and treating diverse sicknesses and perceptions with respect to their pharmacological actions.

South China is one of the most dominant jute-producing regions in China, and huge diversity in available germplasm exists. However, screening out the genotypes based on bioactive compounds and antioxidant potential as well as breeding efforts so far has been restricted to development of genotypes suitable for industrial and medicinal uses. Therefore, the variations in different phytochemical contents and antioxidant activities of leaves from *Corchorus* sp. of different populations were evaluated in this study. The present study was undertaken to evaluate different phytochemical components such as total flavonoids, polyphenols, saponins, tannins, and three minerals, moreover, remarkable contributions to antioxidant capacity, and picked up most desirable populations containing the best yield of antioxidant. To the best of our knowledge, however, no research has been undertaken in order to determine the contents of saponin and tannin from jute leaves. Thus, this study is the first one that has focused on saponin and tannin content from jute leaves. 2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant potential (FRAP), and the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay were used to measure the antioxidant capacities of leaf extracts.

## 2. Materials and Methods

**2.1. Plant Material.** *C. capsularis* and *C. olitorius* were planted on an experimental farm on 15 May 2018 under the natural condition of Changsha, Hunan (Table 1). The fresh top 3 leaves were collected 45 days after sowing (DAS), and each sample contained a minimum of three individual plants of similar genotypes that were mixed to form one sample. The leaves were then allowed to dry under room conditions for 10 days. After drying, the leaves of each genotype were ground to make powder, tagged for identification, and kept in airtight polythene at  $-20^{\circ}\text{C}$  until used in experiments.

**2.2. Chemicals and Reagents.** Potassium acetate ( $\text{CH}_3\text{COOK}$ ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu, metaphosphoric acid, and sulfuric acid 98% (w/w) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) of analytical grade, nitric acid ( $\text{HNO}_3$ ), perchloric acid ( $\text{HClO}_4$ ), and ethanol were collected from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Standard flavonoid compound rutin (>98%), tannic acid, and tea saponin (96 wt.% purity) were ingathered from Tianjin Section Chemical Co., Ltd (Tianjin, China). Vanillin (AR)

TABLE 1: Origin of 30 different genotypes of *C. capsularis* and *C. olitorius*.

Code	Species	Cultivar	Origin (province/country)
C1	<i>C. capsularis</i>	Zhonghuama 1	Changsha, Hunan
C2	„	Yueyin 1	Guangdong
C3	„	Taiwanlvguo	Taiwan, Yuanjiang
C4	„	Yuduhuangma	Yudu county, Jiangxi
C5	„	Longxihongpi	Longxi county, Guangdong
C6	„	Yunxiaofenhongpi	Yunxiao county, Fujian
C7	„	Pinghehaoma	Yunxiao county, Fujian
C8	„	Nananhongma	Nan'an county, Fujian
C9	„	Zaoanqingpi	Yunxiao county, Fujian
C10	„	Nanjingqingpi	Yunxiao county, Fujian
C11	„	Min 33	Fujian
C12	„	Miandianyuanguo	Myanmar
C13	„	Huangma 971	Hunan
C14	„	Minhuang 1	Fujian
C15	„	Wenzhouqingpi	Wenzhou, Zhejiang
C16	„	Wenzhoutuma	Wenzhou, Zhejiang
C17	„	Zhenong 12	Zhejiang A&F University, Zhejiang
C18	„	Zhenong 19	Zhejiang A&F University, Zhejiang
C19	„	Zhenong 9	Zhejiang A&F University, Zhejiang
O1	<i>C. olitorius</i>	Zhongyinhuangma 1	Changsha, Hunan
O2	„	Mali sy025	Mali
O3	„	Jiangxi Guangfengchangguo	Guangfeng county, Jiangxi
O4	„	Kuangyechangguo	Hunan
O5	„	India TTP	India
O6	„	Funong 6	Fujian A&F University, Fujian
O7	„	T8	Zhejiang
O8	„	Maliyengshengchangguo	Mali
O9	„	Zhonghuama 5	Changsha, Hunan
O10	„	Funong 5	Fujian A&F University, Fujian
O11	„	Zhejiang 5	Zhejiang

was bought from Damao Chemical Reagent Factory (Tianjin, China). 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and gallic acid were bought from Aladdin Chemistry Co., Ltd. (Shanghai, China). All solvents and reagents were of analytical standard, and all aqueous solutions had been made from the utilization of freshly double-distilled water. Single-stock solutions of Mg, Zn, and Se (1000 µg/ml) were obtained from ULTRA Scientific (North Kingstown, USA).

**2.3. Quantification of Total Flavonoid Content (TFC).** For total flavonoid content (TFC) determination, samples were extracted using the reflux method for 2 h at 80°C with 80% (v/v) ethanol with 1 g: 25 ml (solid: liquid ratio). The digested solutions were centrifuged at 8000 × g for 4 min. Supernatants were then pooled in clean flasks, and the procedure was repeated. After centrifugation, supernatants were taken out in the flask, with final volume up to 100 ml by 80% ethanol.

The amounts of TFC in the sample were assessed following a modified aluminum chloride assay method outlined by [22]. Briefly, 1 ml (containing 10 mg/ml) extract of each genotype was placed in a 10 ml volumetric tube. First, 2 ml of 0.1 M AlCl<sub>3</sub> was added to every volumetric flask and incubated for 5 min. After that step, 3 ml of 1 M CH<sub>3</sub>COOK was added; the volume brought up to the 10 ml mark with 80% ethanol and then mixed well. After 10 min of

incubation, absorbance was recorded at 420 nm in the tube containing the reagent control, and as standard rutin was used. The results in the sample extract were determined from the standard calibration curve ( $Y = 0.028x - 0.011$ ;  $R^2 = 0.997$ ) that was constructed by plotting absorbance against different rutin concentrations. Results were disclosed as rutin equivalents (mg RE/g dry weight). All determinations were executed in triplicate.

**2.4. Measurement of the Total Polyphenols.** In the determination, 1 g of dry powder from each sample was standardized in 80% (v/v) ethanol (10 ml) and refluxed for 2 hours at 80°C temperature. The supernatant was then collected in a 100 ml volumetric flask and diluted up to mark. This supernatant also was used for investigation of the total tannin content.

Total polyphenols in the samples were investigated by conducting the Folin-Ciocalteu (FC) colorimetric technique proposed by [23] with slight modifications. Briefly, 0.5 ml of the extracted plant samples was pipetted into a test tube and diluted with 1.5 ml distilled water. FC (0.2 ml) was then added and subsequently, the mixture was gently oscillated and held at room temperature for 4 min. After that, 0.8 ml of freshly made 10% (w/w) Na<sub>2</sub>CO<sub>3</sub> was added to each sample, and the mixture was then vortexed. After incubation at 1 h in dark at room situation for the adequate reaction, the absorbance of the mixture was spectrophotometrically at

765 nm against reagent blank. All determinations were estimated in triplicate. Total polyphenol content was computed using standard curve prepared with gallic acid and results showed equivalents of gallic acid (mg GAE/g DW).

**2.5. Determination of Total Tannin Content.** Total tannin estimation was done by using the FC technique described by [24] with modifications. The plant extract (1 ml) was diluted with 49 ml of distilled water, 0.1 ml metaphosphoric acid, 1.7 ml 75% ethanol, 2.5 ml of FC, and 10 ml of (1.0 mol/ml)  $\text{Na}_2\text{CO}_3$  in a 100 ml volumetric flask. The mixture was shaken properly and kept for 15 min at room temperature. The absorbance of sample mixtures and standard solutions were measured against the blank in a spectrophotometer (UV 2700, Shimadzu, Japan) at 680 nm. As a reference, tannic acid (TA) was used, and therefore the total tannin content in the plant extract was reported as equivalent of TA (mg TA/g DW) based on standard curve ( $R^2 = 0.9972$ ).

**2.6. Determination of Total Saponins.** In this assay, heat reflux extraction was accomplished as previously described by [25], with slightly change. For extraction, 1 g powder of each sample was mixed with 50% (v/v) ethanol (20 ml) and incubated 2 h at 80°C for the reflux procedure. The whole extraction was performed twice under the same conditions, and the resulting supernatant was combined and made up to 100 ml with 50% ethanol.

Total saponin in the plant extract was evaluated by vanillin and concentrated sulfuric acid colorimetric approach described by [25]. Briefly, 0.1 ml of the plant extract was combined with 0.5 ml of 50% ethanol, 0.5 ml of freshly made 8% (w/v) vanillin solution, and 4.0 ml of 77% (w/w) sulfuric acid. The mixture then warmed in a water bath to 60°C for 15 min, and after cooling to room temperature, absorbance was determined with an UV/Vis spectrophotometer at 545 nm. The total saponin content in the samples was quantified based on a calibration curve of tea saponin and expressed as mg TSE/g DW.

## 2.7. Determination of Zn, Mg, and Se

**2.7.1. Sample Digestion.** All collected samples were digested. Approximately 0.1 g from each example was used for the digestion process. The weighed sample was added with 10 ml of 100% nitric acid ( $\text{HNO}_3$ ), and 0.5 mL of perchloric acid (100%  $\text{HClO}_4$ ). The mixture was digested for 4 h in a microwave digester under a total pressure of 20 bars and temperature of 220°C in a precise temperature controlled porous digestion furnace (DigiBlock EHD36, Lab Tech). The digestion mixture was heated with an electric hot plate at 200°C until the liquid underwent vaporization. White fumes from the  $\text{HClO}_4$  indicated the end of the digestion method. After cooling, digested samples were adjusted to 25 ml with deionized water prior to analysis. This digestion process was reported previously by [26]. All digested samples had been kept in polyethylene bottles and placed in the refrigerator until used for experiments.

**2.7.2. Mineral Content Determination.** Minerals determined in all samples were magnesium (Mg), zinc (Zn), and selenium (Se). Standard curves with different concentrations were prepared for all of the elements. Obtained results were expressed as mg/100 g of Mg or Zn, while  $\mu\text{g}/100\text{ g}$  was used for Se.

Concentrations of two Zn and Se points from the digested plant sample solutions were evaluated using inductively coupled plasma coupled plasma mass spectrometry iCP RQ iCP-MS (Thermo Fisher Scientific, Germany). Samples were analyzed in thrice. ICP-MS operating conditions consisted of several sets of parameters as follows: power = 1550 W; cool gas drift = 14 L/min; nebulizer gas flow = 1.01 L/min; auxiliary gas flow = 0.8 L/min; nebulizer pressure 3.1 bar, dwell time = 0.01 s; peristaltic pump velocity = 40 rpm; spray chamber temperature 2.70°C; and measurement time of each sample = 3 min.

Moreover, the levels of Mg were studied using FAAS (Model 3110; Thermo Scientific, UK) equipped with a Ca-Mg cathode lamp (PerkinElmer) and air/acetylene flame (6 mA) with gas flow at 1.2 L/min, burner length 100 mm, and a burner height of 17.9 mm. Diluted solutions of the digested plant samples were then fed into a 10 cm air acetylene burner in order to measure the absorbance of the elements at their resonance wavelengths. Instrumental conditions were applied following manufacturer's directions.

**2.8. Antioxidant Tests.** Antioxidants are compounds that can prevent oxidative damage to lipids or different molecules by preventing oxidizing chain reactions. The intensity of antioxidant activity studies of medicinal plants has been rapidly increasing because of the increased amount of attention to their capabilities for uses as natural antioxidants. Different factors, such as genetic factors, weather conditions, polyphenolic compounds, and different secondary metabolites like water-soluble vitamin C and carotenoids, are also related to antioxidant action [27]. Antioxidant capacity is ruled by the various mechanisms of action originating from their antioxidant components. Thus, it has been suggested to use a variety of methods to pertaining to the respective mechanisms. Total flavonoid extracts were analyzed using several *in vitro* test procedures, such as DPPH, ABTS, and FRAP, which are normally used for evaluating antioxidant performance.

**2.8.1. DPPH Radical Scavenging Assay.** The DPPH assay is a straightforward, acceptable and widely used approach for assessing the unconventional scavenging efficiency of plant extracts. The DPPH scavenging assay of sample extracts was quantified according to the method delineated by [28]. In addition, the antioxidant to a DPPH solution in methyl alcohol causes a color/absorbance change in the reagent. The antioxidant compound also has the potential to scavenge the radicals of DPPH, thus causing a color change in the DPPH. Briefly, 2 ml of a newly prepared methanol solution of DPPH (0.05 mg/ml) was added to different concentrations of extracts. The mixture was vigorously shaken and incubated

for 10 min at room temperature until a steady absorbance was achieved. After that point, the absorbance of reduced DPPH radical was measured at 519 nm against a methanolic blank. In general, a lower absorbance value indicates the higher radical scavenging capability. The percentage of free radical scavenging capacity was computed according to the formula-% Radical Scavenging capacity =  $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}}] \times 100$ . The concentration of extract leading to a 50% reduction in DPPH color ( $\text{IC}_{50}$ ) was measured from the linear calibration curve for the extract concentration and percentage inhibition. The  $\text{IC}_{50}$  value indicates the concentration of the sample that scavenges 50% of the unbound DPPH radicals.

**2.8.2. FRAP Scavenging Activity Assay.** FRAP is based on determination of the inhibitor potentials of the samples through the reduction of the  $\text{Fe}^{3+}$ -2,4,6-tripyridyl-s-triazine (TPTZ) in the presence of antioxidants following the procedure narrated by [29]. The working solution of FRAP was made by adding 300 mM sodium acetate buffer (pH 3.6), TPTZ solution, and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution in a ratio of 10 : 1 : 1 in volume. The working solution was warmed at 37°C, and the reactions were carried out within 2 h. After mixing 5  $\mu\text{l}$  of the samples and 180  $\mu\text{l}$  of the FRAP solution were kept at room condition for 10 min. Afterward, samples were read at 593 nm against the blank without sample using a microplate reader (BioTek Instruments, Highland Park, USA) for antioxidant capacity. A freshly prepared  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  working solution was used as standard and results were expressed as mM Fe (II) per g of dried extract.

**2.8.3. ABTS<sup>+</sup> Radical Scavenging Activity.** Determination of the ABTS radical scavenging capability of the sample extracts was based on the reduction of  $\text{ABTS}^{\bullet+}$  radical ion using the hydrogen-donating antioxidant method described by [30]. The ABTS assay was carried out with the business kit directions. Briefly, 10  $\mu\text{l}$  of plant extract and 20  $\mu\text{l}$  of peroxidase solution were mixed with 170  $\mu\text{l}$  of ABTS resolution and incubated at room temperature for 6 min. The reduction of the blue-green ABTS radical was measured at 405 nm with a Spectra Max microplate reader. ABTS activity was expressed as trolox equivalents (mmol TE/g DW), according to the equation received from the standard trolox graph.

**2.9. Statistical Analyses.** SPSS version 16 was used for statistical analyses. All analyzed secondary metabolites, minerals, and antioxidant activities were presented as mean value  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was carried out followed by a least significant differences (LSD) test, and significant differences between data ( $p < 0.05$  and  $p < 0.01$ ) were noted. Moreover, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were achieved with origin 2019 version to pursue the interrelatedness between genotype and cluster characteristics. The dendrogram was obtained with the cluster method of Ward and the squared Euclidean distance

was considered as a coefficient of similarity. In order to interpret and evaluate the relationships between different bioactive components and the total antioxidant potency in *C. capsularis* and *C. olitorius*, a two-tailed Pearson's correlation coefficient evaluation was performed using SPSS 16.

### 3. Results and Discussion

**3.1. Total Flavonoid Content (TFC).** It has been previously described that flavonoids are secondary compounds with antioxidant capacity, and the efficiency of that relies upon the quantity and position of free OH groups [17]. Our results revealed that total flavonoid content varied significantly among the genotypes represented in Figure 1, ranging from 3.04 to 7.89 mg RE/g DW in *C. capsularis* and 5.26 to 13.66 mg RE/g DW in *C. olitorius*. Genotype O7 was shown to contain the highest amount of total flavonoids, whereas the bottom level was determined in C3. Other populations with high flavonoids detected are C10, C15, O2, O4, and O6. These consequences indicate that the total flavonoid content of both species was substantially influenced by the populations of each species. The content of total flavonoid in our current study was 2-3-fold higher than the Tunisian *C. olitorius* as reported by [16]. It has been reported that geographical and climatic conditions, genetic and biological diversity, and seasonal variations are most responsible for affecting the flavonoid contents in plants [6, 31].

Some studies have suggested that flavonoids are associated with reducing the risk of hypertension and cardiovascular diseases, and prevention of the neurodegeneration associated with Alzheimer's and Parkinson's diseases [17]. Day by day flavonoid gaining much popularity as cosmetic ingredients due to capability of protecting skin from ultraviolet light and oxidative stress in order to enhance skin appearance [32].

**3.2. Total Polyphenol Content (TPC).** The results obtained indicated significant differences in phenolic contents between the 30 analyzed cultivars and are presented in Figure 2. The value in *C. capsularis* and *C. olitorius* varied from 5.12 to 7.61 mg GAE/g and 5.41 to 7.78 mg GAE/g DW, respectively. Among the different populations, it was observed that C2, C7, C11, O3, O6, and O7 had the highest total phenol contents, while C16 showed the lowest content (5.12 mg GAE/g of DW) in *C. capsularis* and O10 for *C. olitorius* with a value of 5.41 mg GAE/g. Comparable outcomes of phenolic compounds were obtained by [16], and high contents in this present study were slightly lower than those reported in the literature. This discrepancy may be due to the existence of various amounts of sugars, ascorbic acid or carotenoids or the duration, extraction strategies, or geographical variation, which might also regulate the formation of phenolic aggregates [33]. As reported in a previous study, compared with other plant species, jute leaves have much higher amounts of total polyphenol contents in *Paederia foetida* leaves containing 4.96 mg GAE/g [34], and 109.21–116.70 mg/100 g in sorghum [35]. Polyphenolic extracts are gaining a lot of

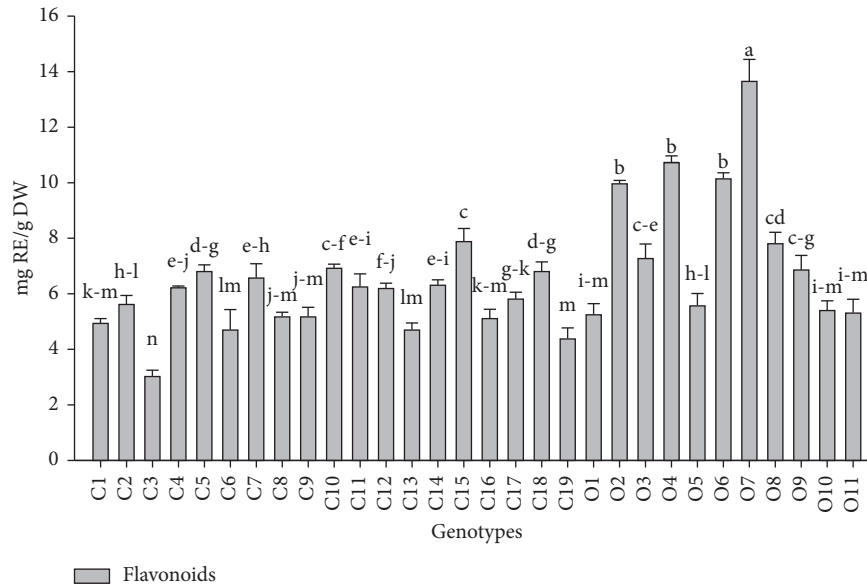


FIGURE 1: Variation in the content of total flavonoids.

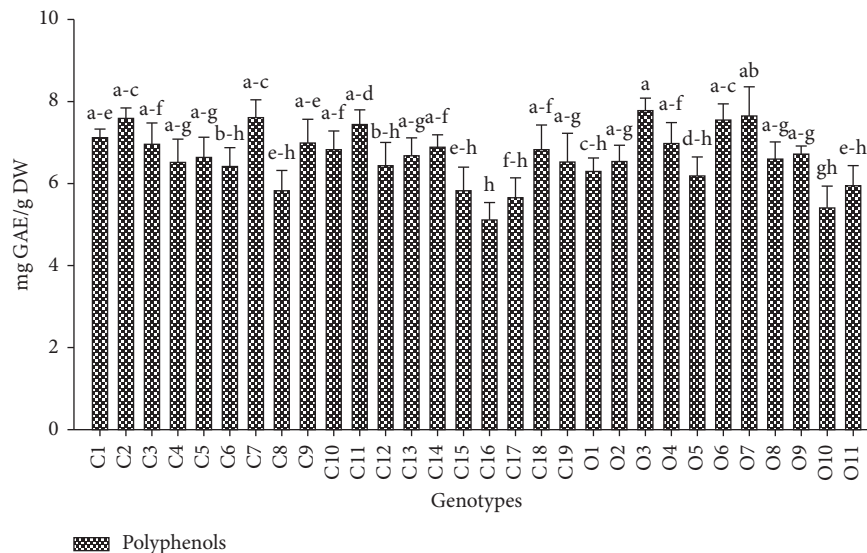


FIGURE 2: Variation in the content of total polyphenols.

attention as active ingredients in cosmetics and medicinal uses due to their high antioxidant properties and capability to extenuate oxidative of extenuating oxidative lipid deterioration.

**3.3. Total Tannins Content (TTC).** Tannins are a unique group of polyphenolic secondary metabolites allotted in higher plants. These compounds possess the capability of complexing strongly with carbohydrates and proteins [36], which can have a considerable influence on the alimentary levels of various foodstuffs utilized by humans and other animals. Total tannins contents of leaves of different genotypes were analyzed and results are presented in Figure 3. Among all genotypes of both species, total tannin contents

ranged from 13.08 to 26.95 mgTA/g. The results displayed that the best results of tannins were observed in C1, C15, O3, O4, and O8, whereas the lowest level was found in C11, C13. The current outcome was much higher than previous results concerning tannin content in the leaves of *P. foetida* with a value of 1.733 mg TAE/g for fresh leaves and 4.96 mg TAE/g for shade-dried leaves [34] and 4.40–26.68 mg catechin/100 g in rambutan seeds [37]. This comparison indicates that jute leaves are an excellent source of tannins. Phytochemical and phytotherapeutic values of medicinal plants are largely influenced by tannins. It has been proven that consuming tannins from plant source may be beneficial to diabetic people because tannins reduce blood glucose levels, scavenge free radicals, and activate antioxidant enzymes in vivo [38]. Since tannins are antifungal, antibacterial, and antiviral

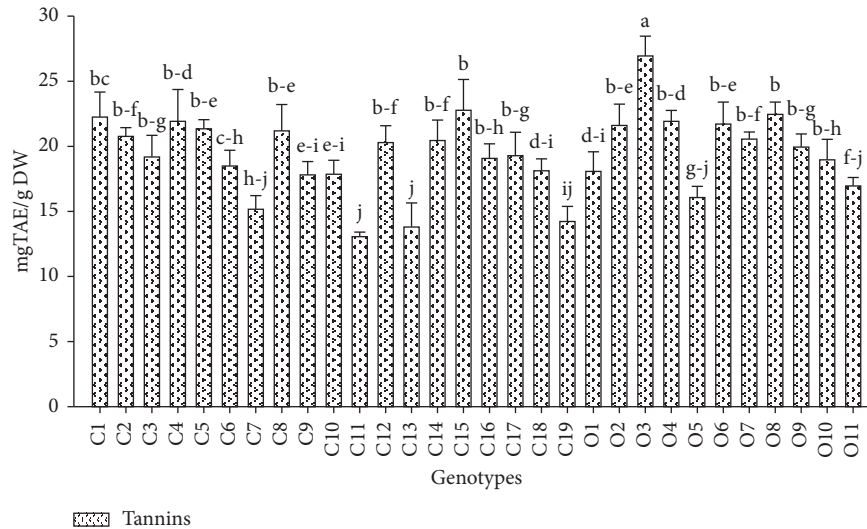


FIGURE 3: Variation in the content of total tannins.

agents, promoting production of jute high in tannins would be useful for in a wide range of medicinal applications.

**3.4. Total Saponin Content (TSC).** Saponins are secondary metabolites of high-molecular-weight glycosides synthesized by many different plants and possess several biological properties [31]. Researchers have found that saponins exist in many herbal medicines as active ingredients and have demonstrated their input to the healthy properties of different foods [39].

The saponin contents of different genotypes of *C. capsularis* and *C. olitorius* leaves are presented in Figure 4. Significant variation ( $p < 0.001$ ) among the different genotypes of jute leaves for the saponin contents in *C. capsularis* ranging from 45.85 to 114.59 mg TSE/g was observed. The highest saponin content was found in C15 followed by C10, while the lowest was recorded in C3 (45.85 mg TSE/g). The saponin content in *C. olitorius* ranged from 34.46 to 92.66 mg/g. Findings showed that the saponin contents in 30 species significantly varied in both species of jute leaves. Another group of researchers reported results of 17.33 and 18.47 mg/g dry weight from *Calligonum polygonoides* and *Zygophyllum album*, respectively [40], which were much lower than results from our study. Saponins are utilized in the preparation of soaps, detergents, fire extinguishers, shampoos, beer, and cosmetics [41]. These findings reveal that it is feasible to use jute leaf saponins in various industries, such as cosmetics. These findings are of relevance because no records on tannin and saponin concentrations in jute had been reported to date. As referred to above, depending on the genotype, jute leaves are an excellent source of nutritional metabolites.

**3.5. Mineral Composition.** The concentration levels of Mg, Zn, and Se of different genotypes of *C. capsularis* and *C. olitorius* are presented in Table 2. The different mineral concentrations were calculated based on the DW of the leaves. Based on the comparison among all genotypes, a

significant difference ( $p < 0.001$ ) from each other was noted. The Mg content in the leaf of *C. capsularis* and *C. olitorius* varied from 33.66 to 37.99 and 34.35 to 37.84 mg/100 g of FW, respectively. Among all genotypes, the highest concentrations of the Mg were estimated in C9 (37.99 mg/100 g), whereas the lowest level was recorded in the C10 (33.66 mg/100 g). The level of Mg in our study was low compared to the 87 mg/100 g as reported by [42] in Africa. Mg is involved in insulin secretion, many oxidative metabolic reactions, reduction of blood pressure, body temperature regulation, nerve impulses transmission, detoxification, energy production, and formation of healthy teeth and bones [43]. Mg is essential and has become the initial treatment in many life-threatening emergency medical conditions.

Zn content in the leaves of *C. capsularis* and *C. olitorius* varied from 0.91 to 7.35 mg/100 g of DW. From both species, it was observed that C7, C11, O2, and O3 accumulated the highest amount of Zn, and lower levels were possessed by C12, and O10 populations. Considering Zn, *C. olitorius* accumulated 21.54 to 37.045  $\mu\text{g/g}$  DW as reported by [42], which is comparable to our results. Our results agreed with the reported findings of other researchers. It has been reported that Zn content in 15 medicinal plants samples presented a mean value of  $0.53 \pm 0.02$  mg/kg [44]. Zn is an essential component of numerous enzymatic reactions, performing an important role in the growth and multiplication of cell and also in HIV positive people. Zn deficiency may lead to loss of appetite, interruption of growth, hair loss, low spirits, delayed wound healing, stagnation in sexual maturation, and emotional disturbances [45].

Se is recognized as a significant micronutrient in humans and animals and its activity seems to be closely associated with the antioxidative attributes of alpha-tocopherol (vitamin-E) and involved in thyroid hormones biosynthesis. The Se content was significantly variable among genotypes of both species, ranging from 85.51 to 168.85  $\mu\text{g}/100$  g DW. Genotypes C5, C7, C8, and C10 showed higher

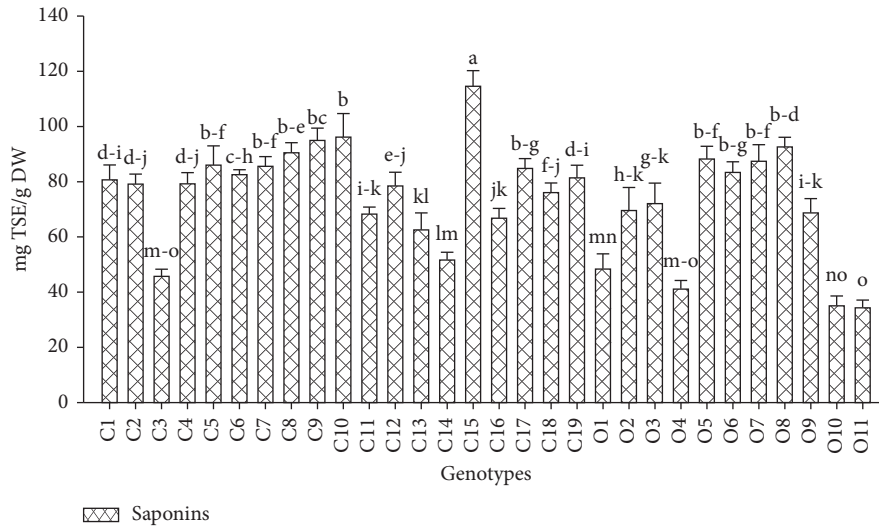


FIGURE 4: Variation in the content of total saponins.

TABLE 2: Mineral content of different genotypes of *C. capsularis* and *C. olitorius*.

Code	Mg mg/ 100 gm $\pm$ SD	Zn mg/ 100 gm $\pm$ SD	Se $\mu$ g/100 g $\pm$ SD
C1	36.82 $\pm$ 1.26 a-e	4.21 $\pm$ 0.80 d-f	111.68 $\pm$ 9.71 j-o
C2	36.56 $\pm$ 0.10 a-f	6.25 $\pm$ 0.51 ab	142.04 $\pm$ 12.54 c-f
C3	36.72 $\pm$ 2.03 a-e	4.69 $\pm$ 0.90 c-e	128.98 $\pm$ 8.83 f-i
C4	37.47 $\pm$ 0.52 a-d	4.75 $\pm$ 0.57 c-e	118.34 $\pm$ 7.20 i-n
C5	37.04 $\pm$ 0.42 a-e	3.45 $\pm$ 0.29 f-h	155.62 $\pm$ 3.87 i-n
C6	36.60 $\pm$ 2.04 a-f	2.65 $\pm$ 0.65 g-j	137.03 $\pm$ 8.70 f-i
C7	36.16 $\pm$ 2.50 a-g	6.53 $\pm$ 1.02 ab	152.18 $\pm$ 8.73 k-o
C8	34.49 $\pm$ 2.89 f-h	5.11 $\pm$ 0.35 cd	168.85 $\pm$ 7.30 e-h
C9	37.99 $\pm$ 0.31 a	2.93 $\pm$ 0.66 g-i	149.00 $\pm$ 12.77 g-j
C10	33.66 $\pm$ 0.51 h	4.67 $\pm$ 0.77 c-e	157.77 $\pm$ 7.98 h-l
C11	35.65 $\pm$ 1.03 c-h	6.56 $\pm$ 1.15 ab	116.68 $\pm$ 5.68 p
C12	36.62 $\pm$ 0.54 a-f	1.16 $\pm$ 0.62 lm	129.76 $\pm$ 9.16 j-o
C13	37.73 $\pm$ 0.18 a-c	5.02 $\pm$ 0.86 cd	107.85 $\pm$ 12.20 k-o
C14	35.08 $\pm$ 2.22 e-h	1.01 $\pm$ 0.32 m	134.15 $\pm$ 8.89 g-k
C15	37.01 $\pm$ 1.06 a-e	2.36 $\pm$ 0.20 h-k	126.02 $\pm$ 8.16 o
C16	37.38 $\pm$ 0.33 a-d	1.16 $\pm$ 0.33 m	121.40 $\pm$ 10.28 no
C17	36.52 $\pm$ 1.51 a-f	3.53 $\pm$ 0.77 fg	85.51 $\pm$ 8.25 h-l
C18	36.52 $\pm$ 1.16 a-f	4.50 $\pm$ 0.83 c-f	113.52 $\pm$ 7.56 m-o
C19	37.07 $\pm$ 0.42 a-e	3.67 $\pm$ 0.98 e-g	109.90 $\pm$ 5.80 l-o
O1	34.35 $\pm$ 2.57 gh	4.38 $\pm$ 0.57 c-f	122.08 $\pm$ 8.97 a-c
O2	37.84 $\pm$ 0.22 ab	6.38 $\pm$ 0.78 ab	116.71 $\pm$ 5.72 d-g
O3	35.77 $\pm$ 1.47 b-h	7.35 $\pm$ 0.61 a	121.13 $\pm$ 13.59 b-d
O4	35.54 $\pm$ 0.70 d-h	5.49 $\pm$ 1.50 bc	99.01 $\pm$ 6.32 a
O5	37.55 $\pm$ 0.17 a-d	3.38 $\pm$ 0.55 f-l	121.24 $\pm$ 11.53 b-e
O6	37.64 $\pm$ 0.98 a-d	4.70 $\pm$ 0.72 c-e	100.87 $\pm$ 9.50 ab
O7	35.50 $\pm$ 0.88 d-h	2.29 $\pm$ 0.35 i-l	105.11 $\pm$ 8.36 i-n
O8	36.84 $\pm$ 0.61 a-e	1.74 $\pm$ 0.17 j-m	121.43 $\pm$ 13.30 h-m
O9	36.48 $\pm$ 0.75 a-g	1.41 $\pm$ 0.31 k-m	105.99 $\pm$ 6.17 op
O10	37.55 $\pm$ 1.15 a-d	0.91 $\pm$ 0.28 m	99.88 $\pm$ 9.21 h-l
O11	36.03 $\pm$ 1.15 a-g	2.56 $\pm$ 0.29 g-j	106.28 $\pm$ 12.69 op

Results are expressed as means  $\pm$  SD of three replicate analyses; different letters mean that they differ significantly at 5% (LSD).

concentrations of the evaluated element in comparison with the other plants. Se concentrations that were determined in different medicinal plants were 0.13–0.92  $\mu$ g/g, and these

results indicated a similar trend as obtained in our study [46]. Due to its antioxidant properties, Se might be capable of halting diabetes progression and its associated complications [47].

Different mineral uptakes by plants are influenced by various factors including types and ages of plant, soil condition, and agricultural and industrial activities [21]. The content of the three elements estimated in this study differed in some cases with literature values due to these factors. It was reported that the considerable mineral concentrations have a large capacity providing different secondary metabolites and minerals [48] that would enhance disease healing processes. Findings from this study offer a quantitative analysis of the phytochemicals likewise mineral elements that are vital for the medical specialties and explain the toxicological deeds of jute plants.

**3.6. DPPH Radical Scavenging Assay.** The DPPH radical assay relies on the speculation that a hydrogen donor is an antioxidant and also that the inhibitor result is proportionate to the disappearance of free radical within the selected sample. DPPH free radical scavenging ability of the various genotypes was disclosed as  $IC_{50}$  values, and low  $IC_{50}$  values appear to be indicative of strong antioxidant capacity.

The  $IC_{50}$  values for DPPH radical scavenging capability were found to be in the range of 247.27 to 351.77 and 206.42 to 303.72  $\mu$ g/ml for *C. capsularis*, and *C. olitorius*, respectively (Figure 5). In *C. capsularis* C11 had low  $IC_{50}$ , indicating that genotype has robust DPPH scavenging capability, whereas C3 showed minimum scavenging activity. In another way, *C. olitorius* O9 revealed excellent antioxidant skills with a value 206.42  $\mu$ g/ml followed by O2, and at a high  $IC_{50}$ , O6 showed weaker DPPH scavenging capacity. A group of researchers reported that  $IC_{50}$  showed less scavenging capacity in *Rhus* species [49] and *Aspalathus linearis* [50] with higher values than those obtained in this study. It was found that an increase in the concentration of total



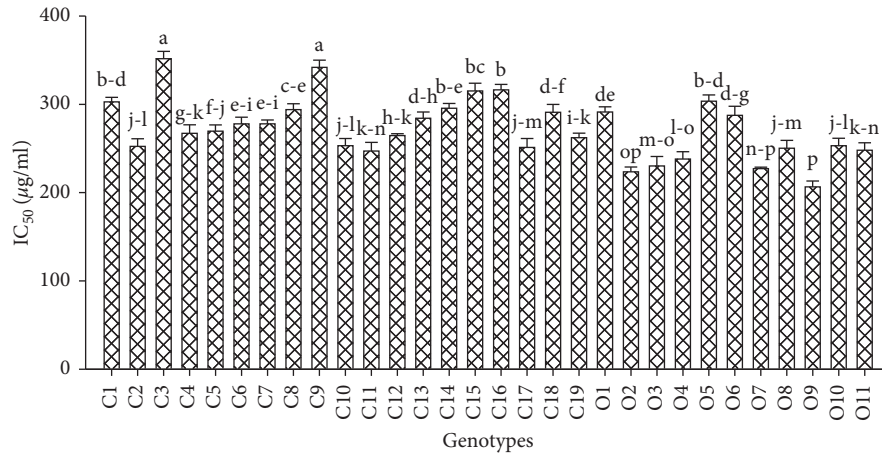


FIGURE 5: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity.

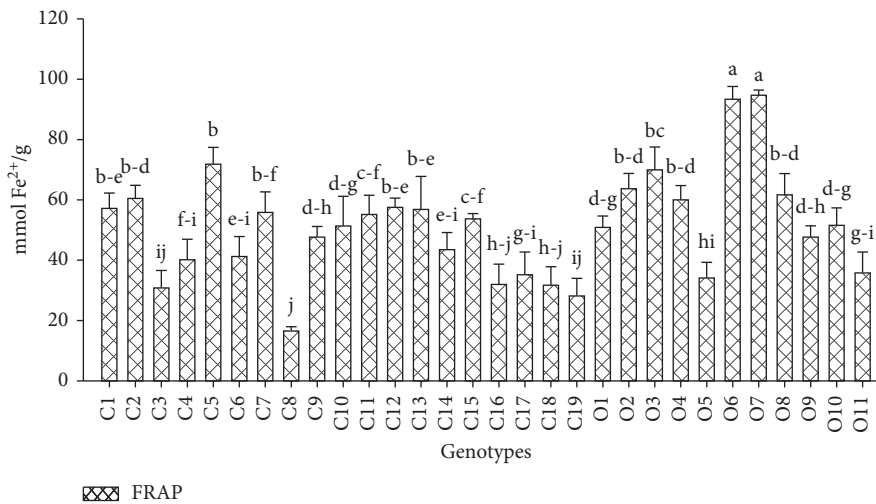


FIGURE 6: Variation of FRAP (ferric reducing antioxidant power).

flavonoids resulted in an incremental change inhibition of the DPPH radical in *Plumula nelumbinis* [51].

**3.7. Ferric Reducing Potential Antioxidant Assay.** It was suggested that most of the secondary compounds are redox-active compounds based on the FRAP assay by the action of electron-donating [52]. The transformation capacity of compounds from Fe<sup>3+</sup>/ferricyanide complex to the Fe<sup>2+</sup>/ferrous form acts as a dynamic indicator for antioxidant activity [53]. Extended absorbance of the reaction mixture implied higher reducing strength. The antioxidant capacity varied from different genotypes of both species, ranging from 16.69 to 94.69 mmol Fe (II)/g DW of the plant (Figure 6). The best possible reducing ability was recorded in C5, O3, O6, O7, and O8 whereas the low-level activity was found in C8, C19, and C3 populations. The FRAP values of the tested leaf extracts were extremely high compared with other natural plants, vegetables, fruits, and fruit wastes [30, 34, 49]. Some studies have illustrated that radical scavenging activity and reducing the potential of antioxidant factors are closely related to total phenolic

content [54], that they are electron donors, and that they can react with free radicals to convert them to more stable products such that they terminate radical chain reactions. The plants with high levels of phenolic content exhibit greater ferric reducing power.

**3.8. ABTS Radical Scavenging Assay.** ABTS is a common test used to investigate the antioxidant activities of plant extracts because of the capability of reducing free radical generation and lowering oxidation stress, in addition to being a quick straightforward technique [55]. In ABTS, scavenging activity of the genotypes varied significantly and ranged from 50.27 to 144.73 mmol TE/g extract for *C. capsularis* and 102.13 to 149.90 mmol Trolox/g extract for *C. olitorius* displayed in Figure 7. By comparison, in the two species, results revealed that the greatest scavenging power was demonstrated in C15, C16, O7, and O8 populations, while C3 demonstrated poor ability. It was reported that high ABTS radical scavenging activity might be due to the presence of phenolic compounds [56]. In cereal and legume flours, their composites, and porridge, ABTS activity reported by [57] was comparable to

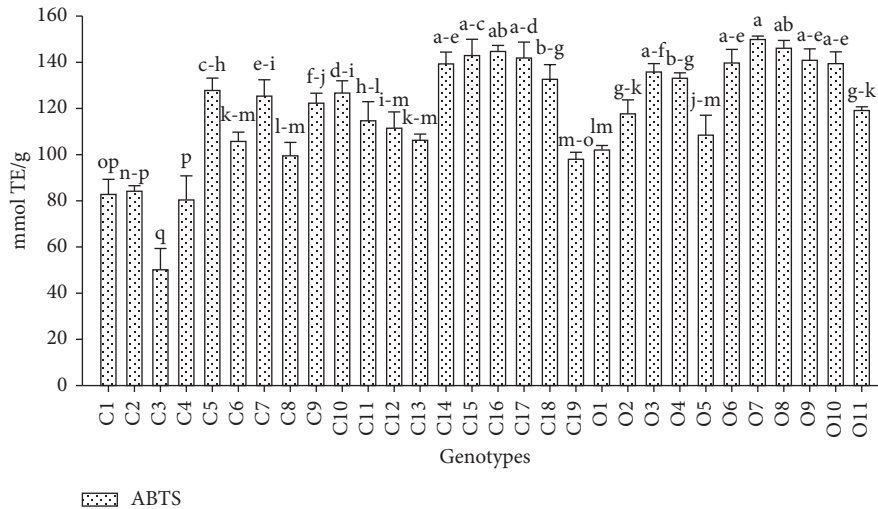


FIGURE 7: Variation of ABTS (2,2-azino-bis 3-ethylbenzothiazolone-6-sulphonic acid) radical scavenging.

our results, but much higher compared to the findings of [58], in which ABTS activity was 1110–3766  $\mu\text{mol TE/g DW}$  in *Hypochoeris radicata*. This result confirms that jute leaf is a potential source of antioxidants for use in the food industry.

The results obtained from different antioxidant studies confirm that jute leaf extracts have the power to scavenge free radicals, thereby inhibiting lipid oxidation by way of a chain breaking reaction, are indicative of high antioxidant properties, and might serve as practical nutraceuticals.

**3.9. Hierarchical Clustering Analysis (HCA).** In the HCA, samples were classified according to Wards method and the squared Euclidean distance in order to evaluate the similarity measure, without taking into account the information about the genotype class. HCA was performed to investigate the correlation among populations and to present similarities of jute genotypes belonging to *C. capsularis* and *C. olitorius* based on measurements of bioactive compounds and antioxidant capacities as presented in the dendrogram in Figure 8.

Based on the secondary compounds, the 30 genotypes were clustered into five groups. Group I consisted of four genotypes C1–C4 accounting for 13.33% of the total genotypes characterized by a low range of flavonoids and minimal DPPH radical scavenging activity, high  $\text{IC}_{50}$ , and low ABTS activity. Cluster II, the largest cluster constructed using 11 genotypes, namely, C6, C7, C8, C9, C11, C13, C14, C18, C19, O1, O5, and O11, accounted for 36.67% of the total populations with low tannin content. From the cluster analysis, a high level of polyphenol content in the cluster 4, which incorporated C7, C11, and C13 genotypes, was observed. Cluster III contained four genotypes (C16, C17, O10, and O11) having the lowest mean value of total polyphenol, saponin, and FRAP (5.54, 55.34, and 38.74, respectively), but interestingly high ABTS power. The last cluster V consisted of O2, O3, O4, O6, O7, and O9 genotypes and was characterized by high mean values of flavonoids, polyphenols, and tannins 9.77 mg RE/g, 7.20 mg GAE/g, and 22.13 mg

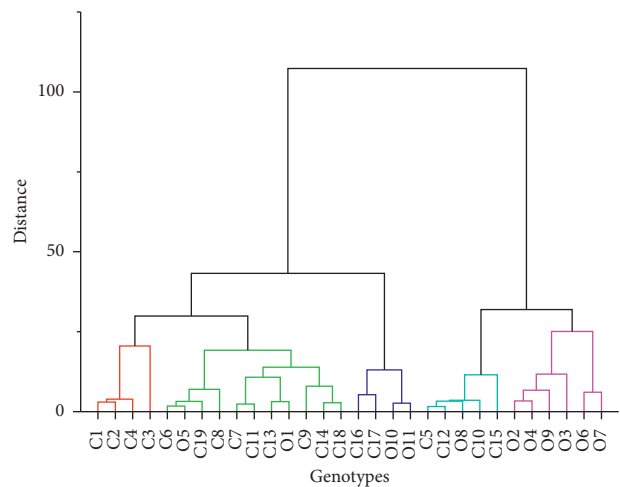


FIGURE 8: Dendrogram of the studied jute genotypes, obtained by cluster analysis (Ward and Euclidean distance) based on secondary metabolites and antioxidant activity.

TAE/g DW, respectively. On the other hand, regarding DPPH scavenging power, cluster V showed the lowest  $\text{IC}_{50}$  for DPPH indicated a robust antioxidant capacity. Besides, Cluster V showed high FRAP activities followed by group IV, whereas poor FRAP activities with the lowest mean value were observed in cluster I. This finding is in agreement with the results of the PCA in which distribution of all genotypes on score plot presented a similar trend.

**3.10. Principal Component Analysis.** PCA is a multivariate exploratory tool that is used to minimize the multidimensional form of the facts and furnish a two-dimensional map to provide an explanation for the determined variance. In the PCA, all major parameters, such as four secondary compounds (TFC, TPC, TTC, and TSC), and the three antioxidant activity measurements (DPPH, FRAP, and ABTS) were introduced in order to categorize the genotypes. The plot summarizes the data from this matrix as principal

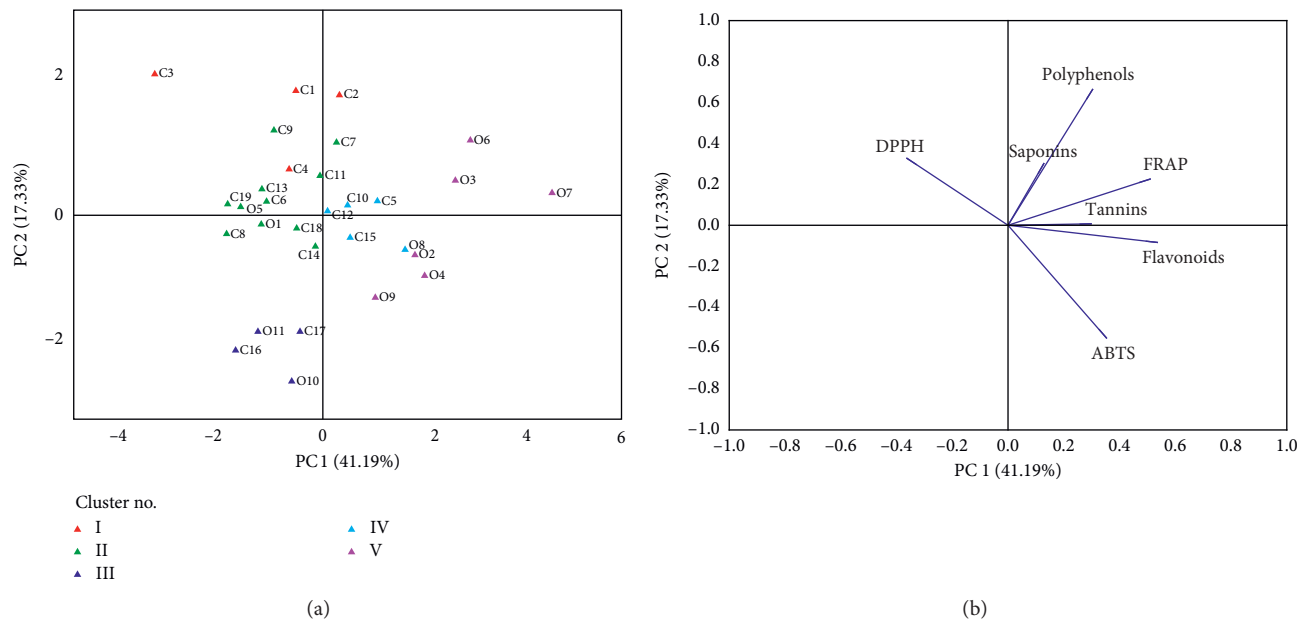


FIGURE 9: Principal component analysis (PCA) plots. (a) The scores plot for different genotypes of *C. capsularis* and *C. olitorius* and (b) the loading plot loading plots for different variables on PC1 and PC2.

components, PC is a linear combination of the original responses, and PCs are orthogonal to each other. The two principal components were determined based on Kaiser Criterion (PCs), with Eigen values > 1 (2.88, 1.21, and 1.11), which were considered significant.

The first two components of the PCA accounted for 58.52% of the total variance (PC1 = 41.19% and PC2 = 17.33%) of the studied parameters. The loading plot of the first two PCs illustrates the main dependence between variables. PC1 correlated mainly with the contents of total flavonoids, total tannins, FRAP, and DPPH, while PC2 correlated with total saponins, total polyphenol content, and ABTS activity. The sample score plot for PC1 versus PC2 is shown in Figure 9. Based on the PCA results and scores, we should be able to distinguish the populations from different groups.

The plot grouped the populations, such as O2, O3, O4, O6, O7, and O9 grouped in V, located right half of the plot, differing considerably from all other classes, according to their phytochemical similarity and antioxidant. This cluster had the best chemical profile of the extracts since it was rich in flavonoids, polyphenols, and tannins and showed excellent antioxidant activities, whereas genotypes C1–C4 were combined together to form group I and were characterized by contrasting properties, indicating that the composition in terms of at least of some of the analytes was measured in addition to its antioxidant activity. All of the other groups had rather small values in comparison with group V. By applying PCA and HCA six vegetables and eight fruits were successfully distinguished into four major groups based on their antioxidant activity [59]. It was interesting to compare the score plots with the HCA, and results revealed that the PCA score plot results were consistent with HCA scores, indicating bioactive compound and antioxidant differences.

**3.11. Correlations Among Phytochemical Compounds.** Pearson's correlation coefficient was performed in order to illuminate the relationship of all of the measured parameters. Different literature reported the Pearson correlations among the secondary metabolites and antioxidant activity in several plant species [60, 61]. Analytical results indicated that the best correlation was between TFC and FRAP (0.731\*\*) as well as between TPC and FRAP (0.590\*\*), followed by TFC and ABTS (0.573\*\*) (Table 3). This correlation of TF with FRAP and ABTS values suggests that the secondary antioxidant metabolites, particularly flavonoids, within the leaf extracts, may also react with free radicals. TPC was a positive correlation obtained with FRAP at the 1% level. Similar trends were reported by [61], who noticed that phenolic content had significant positive correlations with FRAP ( $r = 0.992$ ,  $p < 0.01$ ) in wampee fruits.

Among the minerals, Zn and Se had a positive effect on TPC and TSC at the 5 and 1% level, respectively. The relationship indicated that an increase in Zn content strongly influenced and caused an increase in TPC and Se influences on leaf saponin contents. Correlation analysis revealed that antioxidant activity as evaluated with the FRAP assay showed a positive relationship with the results of TFC and TPC while ABTS and TFC presented at 1% level. Significant positive correlation was also noticed between TFC and TPC, TFC and TTC, and ABTS and FRAP at 5% significance. There were significant negative correlations between TFC and DPPH, ABTS to Zn and DPPH; FRAP and DPPH ( $p < 0.05$ ). By evaluating the correlation coefficients, it is possible to conclude that flavonoids, polyphenols, tannins, and saponins are notably accountable for antioxidant activities of interest in the selected plant species leaf extracts.

TABLE 3: Pearson's correlations among 10 studied traits of *C. capsularis* and *C. olitorius*. (considered variables: TFC, TPC, TTC, TSC, Mg, Zn, Se, DPPH, ABTS, and FRAP).

	TFC	TPC	TTC	TSC	Mg	Zn	Se	DPPH	FRAP	ABTS
TFC	1									
TPC	0.367*	1								
TTC	0.398*	0.095	1							
TSC	0.169	0.125	0.14	1						
Mg	-0.103	-0.159	-0.028	0.1	1					
Zn	0.074	0.550**	-0.009	0.043	-0.164	1				
Se	-0.261	0.111	0.009	0.395*	-0.311	0.15	1			
DPPH	-0.505**	-0.193	-0.157	0.13	0.211	-0.127	0.339	1		
FRAP	0.731**	0.590**	0.353	0.135	0.07	0.14	-0.169	-0.378*	1	
ABTS	0.573**	-0.115	0.181	0.142	-0.054	-0.370*	-0.238	-0.369*	0.367*	1

\*\*Correlation is significant at 1% level, and \*correlation is significant at 5% level.

#### 4. Conclusions

Secondary metabolite molecules in plants food are attributed with various biological activities. The present and definitive findings demonstrate that leaves of *C. capsularis* and *C. olitorius* with the high level of phytochemical constituents include flavonoids, polyphenols, tannins, and saponins that possess strong radical scavenging activity and antioxidant power. Similar conclusions were drawn for PCA or HCA based on data sets. Based on the HCA and PCA, 30 populations belonging to *C. capsularis* and *C. olitorius* effectively formed five distinct groups. Cluster V is strongly recommended for harvesting the leaves in order to obtain bioactive compounds, especially flavonoids, polyphenols, and total tannin contents. Based on antioxidant activities, the result indicated that group V can be considered for extraction of natural antioxidants. Therefore, the genotypes from cluster V could serve as elite material for facilitating breeding strategies to supply a promising potential as an outstanding source of natural antioxidants in food, pharmaceutical, and cosmetics industries.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare no conflicts of interest in this research.

#### Acknowledgments

The authors are very thankful to the Department of Chemistry of the Institute of Bast Fiber Crops for the use of its facilities for this study and also express gratitude to Dr. Liu Liangliang, Professor of Department of Chemistry (IBFC), for his cordial guidance's. This research was conducted and supported by the Central Public-Interest Scientific Institution Basal Research Fund of the Chinese Academy of Agricultural Sciences (GSCAAS), China, Grant number 1610242019002.

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