# Antioxidant Activity and Components of the Ethanol Extract of Sisal Waste

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**Abstract:** *Agave sisalana* can be used as medicinal plant to treat some oxidative stress-caused diseases. This study aimed to fractionate and evaluate the antioxidant components of the ethanol extract from sisal waste. The crude ethanol extract of sisal waste was fractionated into petroleum ether fraction (PEF), ethyl acetate fraction (EAF), n-butanol fraction (BF) and water fraction (WF). Among this different fractions, EAF had the strongest reducing power and radical scavenging capacity with $IC_{50}$ of 40 mg/mL against DPPH· and 870 mg/mL against OH·, respectively, comparable to or even lower than that of BHT. EAF also had the highest content of total phenolic (214.6 mgGAE/g) and total flavonoid (179.8 mgRE/g). HPLC-MS analysis revealed the major components of EAF were flavanols and flavanones compounds.

**Key words:** Antioxidant, free radicals scavenging, ethanol extract, sisal waste.

## 1. Introduction

*Agave sisalana*, generally called sisal, is a monocotyledonous plant of the Agavaceae family. Sisal is a characteristic tropical economic crop and mainly as an important source of hard fiber [1]. Sisal can also be used as an herbal medicine to treat various diseases, especially against those oxidative stress-caused diseases, such as hypertension [2], diabetes and cancer [3]. Sisal waste is the residual after the fiber extraction process, accounting for more than 95% of the sisal mass [4]. According to Zhang's report, the sisal waste is a valuable agricultural residue rich in nutrients and secondary metabolites with multiple functions [5]. Another study has described some extracts from sisal waste possess biological activities such as antimicrobial, antiparasitic larvicidal, and ovicidal [6]. In recent years, many bioactive ingredients have been isolated from sisal waste, such as saponin [7], homoisoflavonoids and flavones [8], pectin [9], and polysaccharide [5].

Although sisal has been traditionally used as a natural functional herb for oxidative stress-related diseases treatment for centuries, there have been only a few reported studies on the antioxidant activity of sisal. According to Ben Hamissa et al, the methanol extract from *Agave americana (L.)* leaves showed strong antioxidant activity and an alternative source of polyphenols [10]. Reference [11] compared the antioxidant activity of the ethanol and hexane extracts from six sisal species cultivated in Mexico and found that the hexane extract of *Agave rzedowskiana* was more active than the ethanol extract. However, reference [12] reported that the lipophilic extracts accounted for 95% and the hydrophilic extracts accounted for only 5%

of the ORAC antioxidant capacity of three common sisal plants. Therefore, most previous studies have suggested that the major antioxidant constituents of sisal were soluble in water or other polar solvents. Compared to water, ethanol has been more favorable and widely used for extracting antioxidant components from natural products with a broad polarity spectrum in previous studies [13]-[16]. This research aimed to assess the antioxidant activity of various fractions from sisal waste and constituents of the fraction with highest antioxidant activity, and to evaluate the potential of sisal waste as a useful antioxidant.

## 2. Materials and Methods

### 2.1. Materials

The sisal waste was collected directly from a decortication machine on Guangxi Nanjian biological technology Co., Ltd, Guangxi, China. 1 Kg sisal waste was cleaned with DI water, oven-dried at 60℃, crushed into meals and passed through a 60-mesh sieve. The dried sisal waste meals were stored for later use. The standard reagents were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Other organic solvents were made in China and of analytical grade.

### 2.2. Preparation of Ethanol Extraction from Sisal Waste

A certain amount of sisal waste was refluxed with ethanol using a solid-liquid ratio (w/v) of 1:20 at 60℃ and the extraction was repeated for 3 times. Then the extraction liquid was combined and concentrated at 50℃ using rotary evaporator under reduced pressure. After drying in vacuum, the crude ethanol extract (CEE) from sisal waste was formed. Calculated based on the gram of CEE per 100 g dried sisal waste, the yield of CEE was 7.7 ± 0.42%.

### 2.3. Fractionation of Sisal Waste Ethanol Extract

As shown in Fig. 1, the obtained CEE was suspended in water and sequentially fractionated with solvents of increasing polarity to obtain petroleum ether fraction (PEF), ethyl acetate fraction (EAF), n-butanol fraction (BF) and water fraction (WF). The obtained fractions were concentrated by a rotary evaporator under vacuum and then dried at 40℃ for 4 h in a vacuum oven. The water fraction WF was concentrated by a rotary evaporator at 60℃ and then freeze-dried. All the dried fractions were kept in brown glass bottle at 4℃ prior to analysis. Calculated based on the mass of each fraction to the total mass of CEE the yield of each fraction was 4.2 ± 0.1%, 12.8 ± 0.2%, 48.7 ± 0.7%, 27.4 ± 0.44% respectively.

### 2.4. Radicals Scavenging Activity Evaluation of Sisal Waste Extract and Its Fractions

The CEE and the extraction fractions were dissolved in 70% ethanol respectively and formulated into different concentrations of solution for radicals' activity determination.

DPPH and OH scavenging activity by different fractions were determined according to [17] and [18] respectively. The radical scavenging rate (RSR) was calculated according (1):

$$\text{RSR}\ (\%) = \frac{A_0 - (A_i - A_j)}{A_0} \times 100 \quad (1)$$

where $A_i$ was the absorbance of the sample or BHT, $A_j$ was the absorbance of the sample with the anhydrous ethanol instead of DPPH or OH, $A_0$ was the absorbance of the control.

The $O^{2-}$ scavenging activity (SASC) of ethanol fractions was measured by pyrogallol methods [19]. The SASC was calculated according to (2):

$$\text{SASC}(\%) = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100 \quad (2)$$

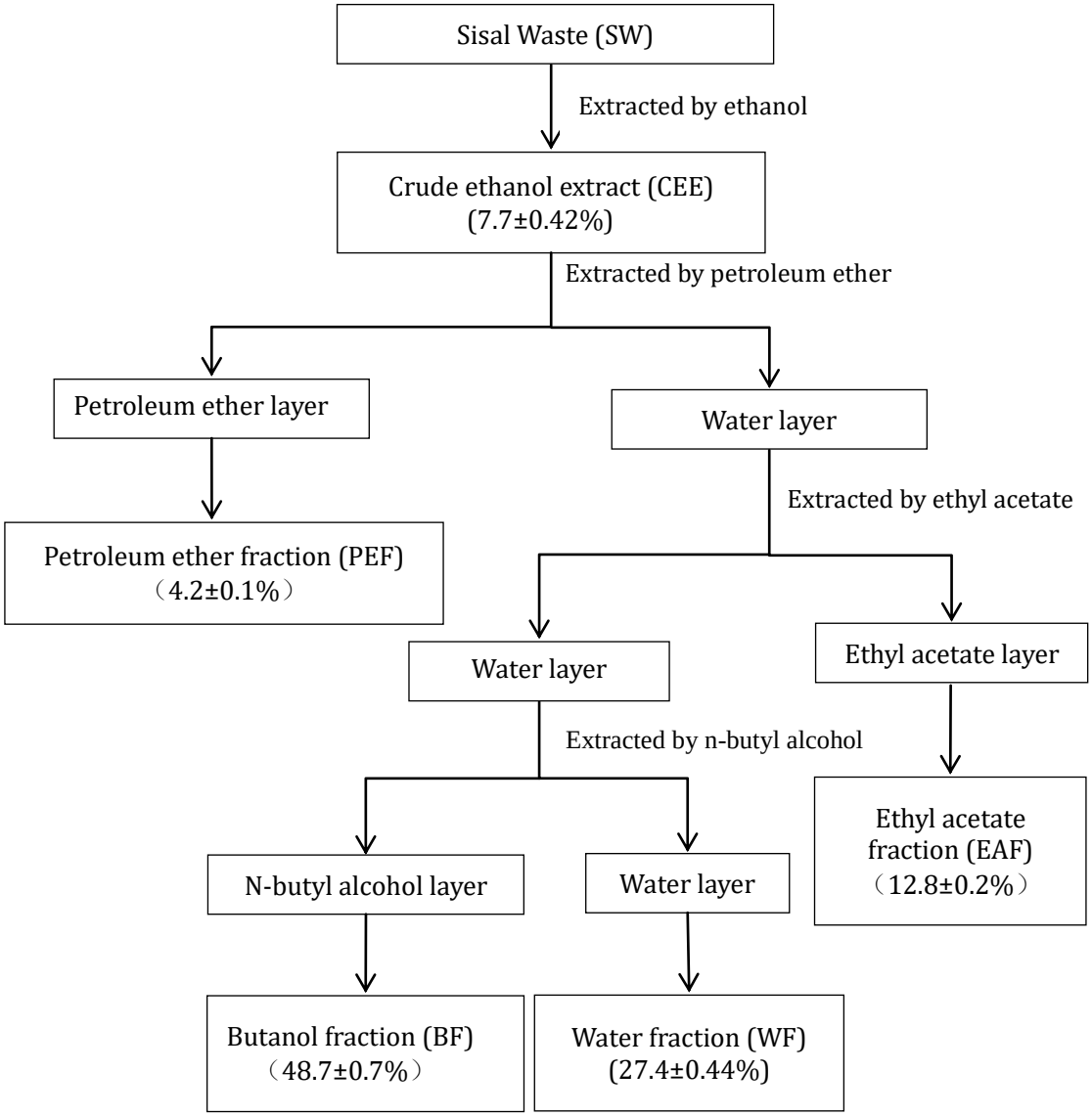


Fig. 1. The schematic process of extraction and fractionation of chemicals from sisal waste.

The absorbance $A_x$ was measured at 325 nm. The solvent was used as the model control group ($A_0$), and the solvent instead of pyrogallol solution was used as the sample control group ($A_{x0}$).

$IC_{50}$ value was obtained from the linear regression equation prepared from the concentrations of the sample and the percentage inhibition of radical scavenging activity. [13]

## 2.5. Determination of Total Phenols Content, Total Flavonoids Content and Total Saponins Content

The total phenols content, total flavonoids content and total saponins content was respectively determined by the Folin-Ciocalteu assay [14], the Sodium Nitrite-aluminum Nitrate colorimetric method [16] and the Vanillin-sulfuric acid method [20]. The results are mean values of three determinations and expressed as per gram of sample gallic acid equivalent (mgGAE/g), per gram of sample rutin equivalent (mgRE/g) and the diosgenin equivalent per gram of the sample (mgSAP/g), respectively.

### 2.6. HPLC-MS Analysis of EAF

EAF, with highest antioxidant activity, was analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS) with a C18 column at room temperature (~25°C). The mobile phase consisted of acetonitrile and methanol, flowing at 1 mL/min and the detection wavelength was 280 nm. The ion source was ESI and the scanning mode was negative ion mode. N2 was used as a drying gas at a constant flow rate of 4 L/min. MS was scanned in a range of 50-1000 m/z. The identification of components was conducted by matching the mass spectra with literature data.

### 2.7. Statistical Analysis

All date were reported as mean ± SD of triplicate-test. Microsoft Excel 2010 was used for linear regression and calculation of the standard deviation. The treatments were statistically analyzed using SPSS 10.0.

## 3. Results and Discussion

### 3.1. Radicals Scavenging Potency of the Ethanol Extract and Fractions

Fig. 2(A) showed the scavenging DPPH activity of ethanol extract from sisal waste and its fractions in comparison with that of BHT. According to the data, significant ($p$<0.05) scavenging of DPPH was evident at all the tested concentrations of SW ethanol extract CEE, fractions PEF, EAF, BF, WF and reference compound BHT (0.02-0.8 mg/mL). The capacity of each fraction to scavenge DPPH increased with the increase of the sample concentration and was in an order of *EAF* > *WF* > *BF* > *PEF*. Meanwhile, the DPPH scavenging ratio by EAF and BHT reached 90%, which could be considered as a full absorption inhibition of DPPH [21]. The ability of EAF to scavenge DPPH was comparable to the positive control BHT at a concentration above 0.2 mg/mL, indicating that EAF was the most effective DPPH scavengers among the five investigated extracts and showed potential to be developed as a natural antioxidant. Another fraction WF was as efficient as BHT at 2.0 mg/mL. While DPPH scavenging activity exerted by BF, PEF and CEE were considerably lower than that by EAF and WF.

Table 1. Total Phenols Content (TPC), Total Flavonoids Content (TFC), Total Saponins Content (TSC) and $IC_{50}$ Values (DPPH, OH, $O^{2-}$) of Crude Extracts and Their Derived Fractions of Sisal Waste

| Fractions | TPC | TFC | TSC | $IC_{50}$ (μg/mL) | | |
|---|---|---|---|---|---|---|
| | (mgGAE/g) | (mgRE/g) | (mgSAP/g) | DPPH· | OH· | $O^{2-}$· |
| CEE | 49.4 ± 2.4 | 68.9 ± 3.1 | 152 ± 4.7 | 160 ± 3.2 | 1290 ± 10.6 | 5200 ± 18.8 |
| PEF | 32.0 ± 2.0 | 22.5 ± 1.6 | 86 ± 2.8 | 260 ± 3.5 | 2790 ± 15.5 | 6740 ± 21.3 |
| EAF | 214.6 ± 4.3 | 179.8 ± 3.6 | 303 ± 6.8 | 40 ± 1.3 | 870 ± 7.9 | 2460 ± 14.4 |
| BF | 64.6 ± 2.9 | 44.4 ± 2.1 | 557 ± 10.2 | 150 ± 2.6 | 1040 ± 9.1 | 4900 ± 18.9 |
| WF | 100.8 ± 3.5 | 53.5 ± 2.5 | 112 ± 4.4 | 80 ± 1.8 | 1060± 10.8 | 4490 ± 17.6 |
| BHT | - | - | - | 12 ± 0.9 | 1280 ± 9.5 | 1520 ± 8.5 |

All the values are mean ± SD; SD: standard deviation.
- : not tested.

The OH scavenging activity of ethanol extract from sisal waste and its fractions was shown in Fig. 2(B). As similar observation in DPPH radical-scavenging assay, all the tested fractions and positive control showed significant OH scavenging activity ($p$<0.05) in a concentration-dependent manner. At lower concentration (0.2-1.0 mg/mL), the OH scavenging rate of the tested samples showed no significant difference ($p$<0.05) except for PEF. While the OH scavenging of EAF and WF reached 81.4% and 72.6% at the concentration of 2.0 mg/mL respectively, indicating that EAF and WF presented higher capacity of hydroxyl radical-scavenging activity than BHT. The rest fractions presented lower activity, less than 60% OH scavenging rate at 2.0 mg/mL.

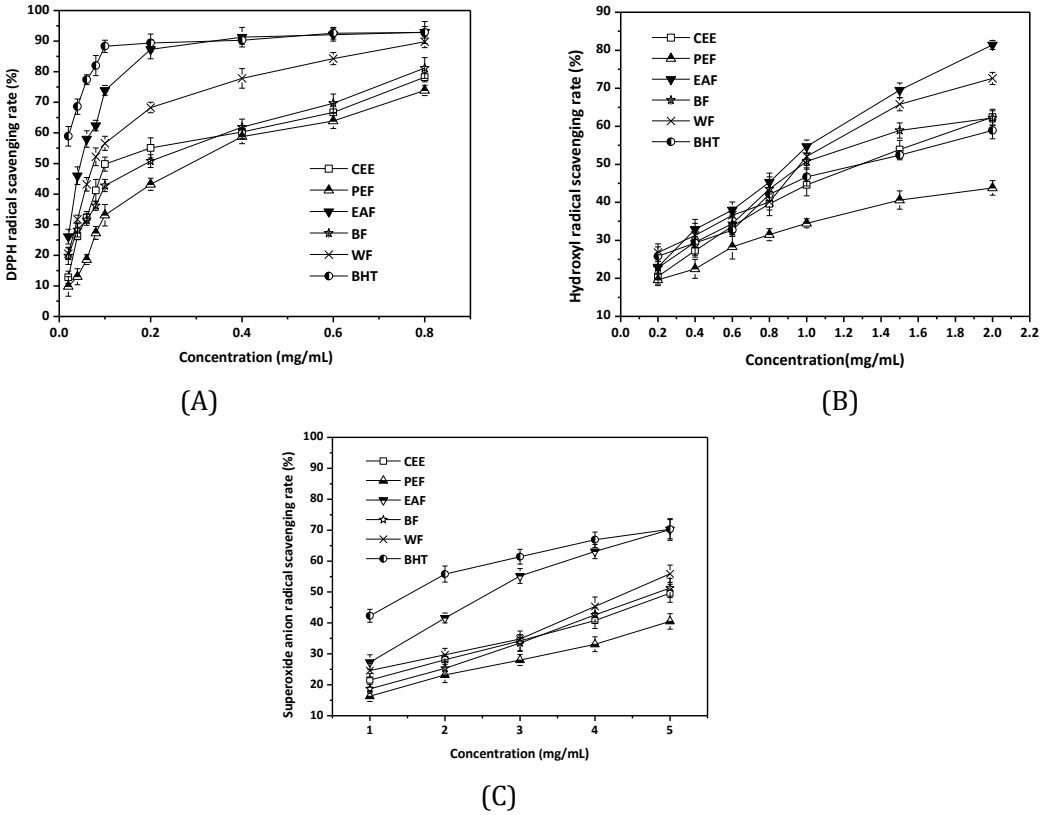


Fig. 2. DPPH scavenging activities (A), OH· scavenging activities (B), $O^{2-}$ scavenging activities (C) of crude ethanol extract from sisal waste and its fractions. Results are presented as mean ± SD (n=3); SD: standard deviation.

Fig. 2(C) depicted the $O^{2-}$ scavenging effect of ethanol extract from sisal waste and its fractions. The results showed that each fraction and BHT possessed the ability of scavenging $O^{2-}$ in a concentration-dependent manner. Obviously, with the increase of the concentration, the scavenging rate of EAF also increased and approached BHT gradually. However, the highest $O^{2-}$ scavenging rate of EAF or BHT was limited to 70%, which was much lower than that of DPPH or OH assay. Similar results have been reported [15], [17]. It may be due to the fact that $O^{2-}$ was not a very reactive radical and thus showed less sensitive to the antioxidants than the active radicals, DPPH and OH. Undoubtedly, EAF was an efficient $O^{2-}$ scavenger and the $O^{2-}$ scavenging ability of EAF was even equivalent to that of BHT at the concentration of 5 mg/mL, which was in accordance with the previous studies on its scavenging effects on DPPH and OH.

$IC_{50}$ value corresponded to greater radical scavenging activity and antioxidant activity. Among all the tested samples, EAF was observed to have very low $IC_{50}$ value of 40 μg/mL against DPPH • , slightly higher than that of BHT. $IC_{50}$ value of EAF against OH was 870 μg/mL, much lower than that of BHT (1280 μg/mL). It further approved that EAF was more effective in OH scavenging that the widely accepted antioxidant BHT.

Similarly, EAF had the lowest $IC_{50}$ value (2460 μg/mL) against $O^{2-}$ among all fractions, but higher than that of BHT. Results demonstrated that EAF had the highest activity of scavenging radicals among the tested sub-fractions derived from the ethanol extract of SW while PEF have the weakest antioxidant activity. Thus, it was speculated that the antioxidant activity of crude ethanol extract from sisal waste was mainly concentrated on the ethyl acetate fraction EAF.

A compound acts as a powerful antioxidant if the value of the $IC_{50}$ against DPPH is less than 50 μg/mL, strong if the $IC_{50}$ value is 50-100 μg/mL, moderate if the $IC_{50}$ value is 100-150 μg/mL, and weak if the $IC_{50}$ appreciating 150-200 μg/mL [22]. Therefore, the ethyl acetate fraction EAF derived from the ethanol

extract of sisal waste could be regarded as a powerful antioxidant.

### 3.2. Contents of Total Phenols, Flavonoids, and Saponins

Table 1 showed the contents of total phenols, total flavonoids, and total saponins in crude extracts from sisal waste and their derived fractions. In the present study, TPC in EAF was 214.6 ± 4.3 mgGAE/g, which was more than two times higher than that of other fractions and CEE. TFC of the fractions followed the order: EAF > CEE > WF > BF > PEF. Meanwhile TSC in BF was much higher than in the order fractions, which supported the findings that such a difference in TPC, TFC and TSC content among the fractions can be explained from the polarity difference of the solvents and the solubility of these components in each solvent. Besides, the order of TPC, TFC, and TSC was in accordance with the radicals scavenging activity of these fractions as described in 3.1 in this paper.

Taken these components content and the antioxidant activities of the fractions together into consideration, we may conclude that the remarkable antioxidant capability of EAF could be mainly associated with its high content of TPC and TFC. Reference [16] indicated that their data underlined the presence of a strong correlation between antioxidant activity and total phenols and flavonoid contents of the ethanol fractions from *Cistus laurifolius L.* leaves. Similar finding was also reported by Yu et al., who studied the antioxidant of various fractions of ethanol extract of Dianthus superbus, and also indicated that the EAF had the highest phenols content and the strongest antioxidant activity [15]. All these findings supported our suggestion that TPC and TSC were likely to be the main contributive components for the strong antioxidant activity of the medicinal plant *Agave sisalana*. TFC represented for flavonoid as one of the most diverse and widespread group natural phenols, which exhibited their antioxidant activity originating from their properties of proton loss, chelate formation, and dismutation of radicals.

### 3.3. HPLC-MS Analysis of EAF

Fig. 3 was the total ion current of ethyl acetate fraction (A) and the ESI-MS spectrum of compounds in ethyl acetate fraction (B). With reference to literature [23]-[26], the possible major constituents of EAF and their m/z were given in Table 2. Among all the identified constituents, many constituents had been confirmed that they are existing in sisal plant, such as 5-hydroxy-7-methoxy-2-trltrlacontyl-4(H)-benzopyran-4-one, tetratriacon-tylhexadecanoate, hecogenin. It was reported that 5-hydroxy-7-methoxy-2-trisatriyl-4-H-chromen-4-one has a strong killing activity against the bacterial Pseudomonas aeruginosa and a moderate antioxidant activity [27]. Hecogenin, belonging to steroidal saponins, had been isolated and was the major constituent of sisal waste [4]. It possessed antiproliferative activity and induction of apoptosis in several cell lines and antioxidant activity, useful in other applications especially as a raw material for the synthesis of other useful pharmaceutical intermediates. In addition, isoliquiritigenin which belongs to flavonoids had a strong antioxidant activity and many pharmacological activities [28]. It was found to be potent antioxidants agents and it was a potential cancer chemopreventive agent. Most flavanols and flavanones had extensive biological activities, including the elimination of radicals, antioxidants, cardiovascular protection, anti-tumor, and prevention and treatment of various diseases. Based on the comprehensive analysis, the antioxidant activity of EAF could have relation with the existence of the phenolic compounds and flavonoids. Of course some other chemical substances also exhibit potential antioxidant activity, such as tetratriacon-tylhexadecanoate, hexadecanoic acid, dibutyl phthalate, behenic acid, etc. It can be observed in Fig. 3 (B) that the major components of EAF from sisal waste are mainly contributed by flavanols and flavanones. However, we suggested that the antioxidant activities of EAF were contributed by different compounds and the strong antioxidant activity of EAF were probably due to the interactions among various components.

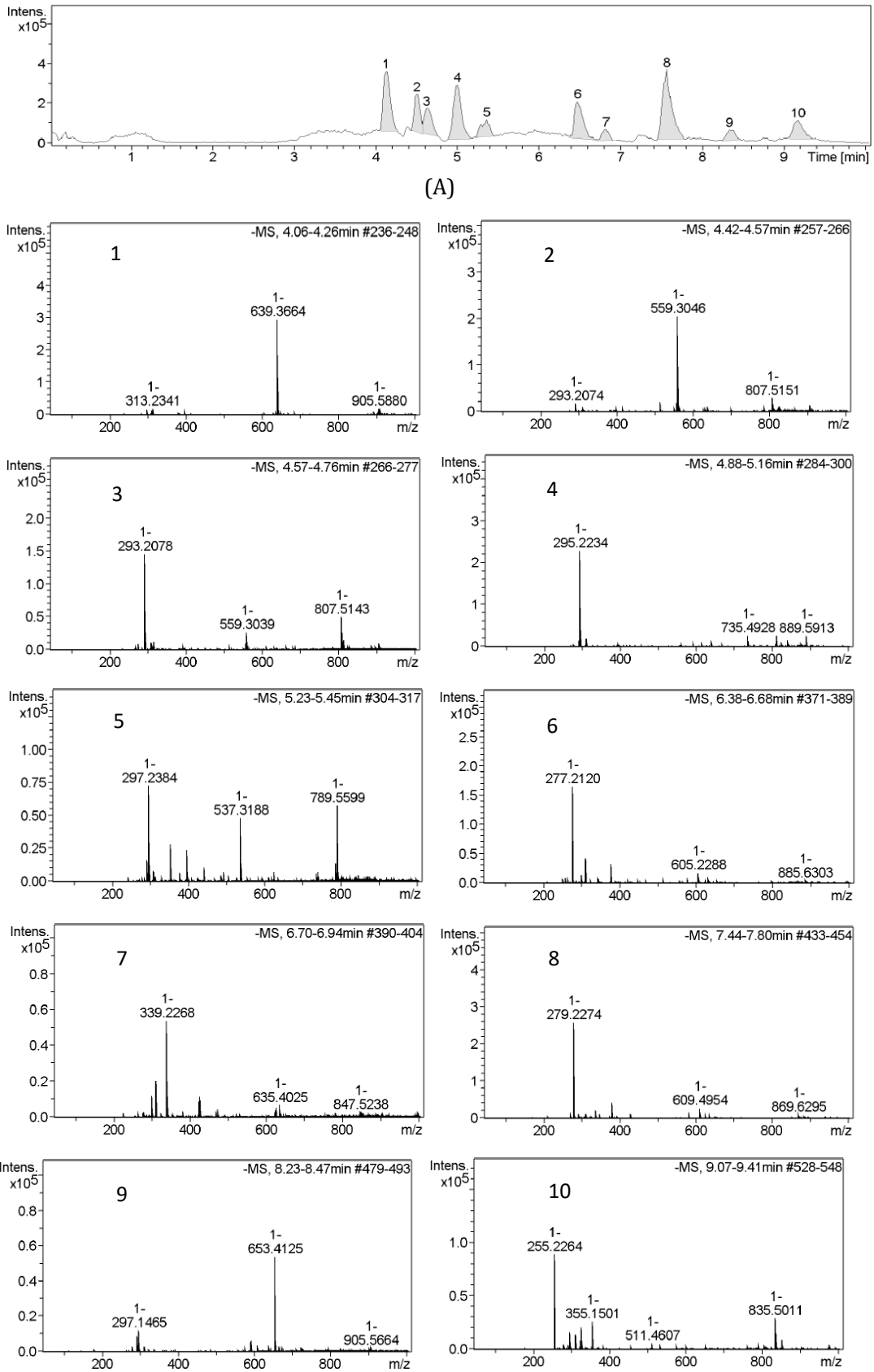


Fig. 3. The total ion current of ethyl acetate fraction (A); The ESI-MS spectrum of compounds in ethyl acetate fraction (B).

Table 2. The Information of Components in EAF

| No. | Retention Time (min) | m/z | Compounds Name | Molecular Formula |
|---|---|---|---|---|
| 1 | 4.13 | 640 | 5-hydroxy-7-methoxy-2-tridecy-4(H)-benzopyran | $C_{43}H_{76}O_3$ |
| 2 | 4.51 | 560 | glycoside formed by β-sitosterol and Rhamnose | - |
| 3 | 4.63 | 294 | 9,12-octadecadienoic acid methyl ester | $C_{19}H_{34}O_2$ |
| 4 | 5.00 | 296 | flavanone or flavanol | - |
| | | 732 | tetratriacon-tylhexadecanoate | $C_{50}H_{100}O_2$ |
| 5 | 5.36 | 298 | Flavanone or flavanol | - |
| 6 | 6.48 | 257 | hexadecanoic acid | $C_{16}H_{32}O_2$ |
| | | 278 | dibutyl phthalate | $C_{16}H_{22}O_4$ |
| 7 | 6.82 | 340 | behenic acid | $C_{22}H_{44}O_2$ |
| | | 430 | tigogenin | $C_{27}H_{42}O_4$ |
| 8 | 7.56 | 280 | flavanone or flavanol | - |
| 9 | 8.33 | 654 | 5-hydroxy-7-methoxy-2-trltrlacontyl-4(H)-benzopyran-4-one | $C_{43}H_{74}O_4$ |
| 10 | 9.17 | 256 | isoliquiritigenin | $C_{15}H_{12}O_4$ |

## 4. Conclusions

In conclusion, the paper studied the antioxidant activities of crude ethanol extract from sisal waste and various fractions, and the components of the ethyl acetate fraction. The EAF of sisal waste showed more effective antioxidant activities than other fractions. Meanwhile, the EAF had the highest content of total phenolic and total flavonoid, and relatively higher content of total saponin. Therefore, our study suggested that the sisal waste can be utilized as a source of potential antioxidants. On the basis of analysis, the antioxidant activities of EAF were due to different compounds and mainly contain flavanones and flavanols. Consequently, our study provided scientific information for the further potential utilization of sisal waste.

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