ABSTRACT
Harnessing the vast supply of underutilized crops would provide several opportunities to increase the temporal and geographical variation in cropping systems, which would ultimately result in a more sustainable supply of a variety of healthy foods for everyday consumption. *Crassocephalum crepidioides* is one of neglected and underutilized vegetables in Nigeria. *C. crepidioides* is a vegetable labelled as poor-man’s food because its medicinal importance and phytochemical profiling is yet to be ascertained. They reputed to be employed in managing variety of ailments such as sores, chest pains, diarrhea, and menstrual cramps, diabetes, inflammation, enteritis. Liquid Chromatography Mass Spectrometry (LCMS) was used to identify the compounds presents in the ethanol extract of the leaves of *C. crepidioides*. The antidiabetic activity of the plant was evaluated employing α-amylase assay while Hydrogen peroxide (H_{2}O_{2}), ABTS and DPPH inhibition assays were employed for antioxidant activity. LCMS shows six (6) compound of different chemical classes and of diverse biological application were identified in the plant extract base on their m/z ratio i.e. hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, alkaloids and other benzoic acids. The ABTS inhibition assay gave the best result with 16.21% at 20 μg/ml though the extract is dose dependent as the activity decreases with corresponding increase in the dosage. This vegetable should not be neglected as the study reveals, *C. crepidioides* contain natural products useful against diseases and ailments and consumed in large quantities in order to have good effects against problems related to oxidative stress.

Keywords: *C. crepidioides*, α-amylase, neglected and underutilized vegetable, LC-MS

INTRODUCTION
By utilizing underutilized crops, temporal and spatial heterogeneity can be introduced into cropping systems, which ultimately leads to a more sustainable supply of diverse and nutritious food for routine consumption. Underutilized crops, previously confined to specific regions, offer a viable means of meeting the food and nutritional needs of a rapidly growing human population. Notably, NUS legumes have an excellent nutritional profile and represent an affordable alternative source of protein, thus, many tropical and subtropical regions such as West Africa, Asia for its medicinal and nutritional properties, renowned for its effectiveness as a remedy for acute hepatitis and fever (Yoko Aniya et al., 2005). The plant thrives in moist areas, natural grasslands, riverbanks, wastage places, roadside and backyard gardens that are rich in organic matters (Burkill, 1995; Tomimori et al., 2012). Its prevalence is particularly noteworthy in tropical Africa and the Okinawa Islands in Japan (Burkill, 1995; Tomimori et al., 2012). *C. crepidioides* is widely grown in Asia for its medicinal and nutritional properties, renowned for its effectiveness as a remedy for acute hepatitis and fever (Yoko Aniya et al., 2005). The plant thrives in moist areas, natural grasslands, riverbanks, wastage places, roadside and backyard gardens that are rich in organic matters (Burkill, 1995; Tomimori et al., 2012). Its prevalence is particularly noteworthy in tropical Africa and the Okinawa Islands in Japan (Burkill, 1995; Tomimori et al., 2012). *C. crepidioides* is widely grown in Asia for its medicinal and nutritional properties, renowned for its effectiveness as a remedy for acute hepatitis and fever (Yoko Aniya et al., 2005). The plant thrives in moist areas, natural grasslands, riverbanks, wastage places, roadside and backyard gardens that are rich in organic matters (Burkill, 1995; Tomimori et al., 2012). Its prevalence is particularly noteworthy in tropical Africa and the Okinawa Islands in Japan (Burkill, 1995; Tomimori et al., 2012). *C. crepidioides* is widely grown in Asia for its medicinal and nutritional properties, renowned for its effectiveness as a remedy for acute hepatitis and fever (Yoko Aniya et al., 2005). The plant thrives in moist areas, natural grasslands, riverbanks, wastage places, roadside and backyard gardens that are rich in organic matters (Burkill, 1995; Tomimori et al., 2012). Its prevalence is particularly noteworthy in tropical Africa and the Okinawa Islands in Japan (Burkill, 1995; Tomimori et al., 2012).
funnel, Whatman filter paper, measuring cylinder, hand gloves, masking tape, beakers, spatula, syringes and weighing balance.

**Collection, Identification and Drying of the Plant**

The leaves of *Crassocephalum crepidoide* were collected in November 2021, at Ilorin, Kwara State, Nigeria. It was identified at the Department of Life Sciences at the Federal University of Dutsin-Ma, Katsina. In the laboratory, the leaves were cleansed with water and air dried for two weeks. They were ground with a pestle and mortar. The powdered samples were maintained at room temperature in clean, airtight containers until they were needed.

**Extraction and Concentration**

Ethanol was used to extract the powdered leaf sample. Ethanol was utilized as the extraction solvent for a period of 7 days on 1000g of powdered leaf sample packed in Bama bottles. The solvent was collected by rotary evaporator at the end of the period. The extract was fractionated using a separation funnel and Hexane as solvent, the polar and the non-polar fraction were collected. After that, the extracts were placed in a desiccator and allowed to dry fully before being tested.

**Alpha amylase inhibitory activity**

α-Amylase inhibitory ability of the extract was decided, employing a reported method though by Zhang et al. (2011).

2. 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity.

The 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonate, ABTS radical cation decolorization assay based on the scavenging of ABTS•+ radicals by antioxidants component of the extracts was used. The assay follows the procedure of Atolani et al. (2013), with slight modifications (Atolani et al., 2013). All analysis was determined in duplicate.

**Liquid Chromatography Mass Spectroscopy (LC-MS)**

The diluted ethanol extract (1 mg/mL) was used for LC-MS/MS analysis. LC-MS triple quadrupole mass spectrometer (Shimadzu 8040) was the main part of the LC-MS/MS system. The ionizations were detected by ESI. The binary pumps (LC-30AD), a column oven (CTO-10ASvp) degaser (DGU-20A3R), and auto sampler (SIL-30AC) were integrated to the LC system. The chromatographic separation was performed on a C18 (150mm x 4.6mm, 3µm) reversed phase analytical column (Inertsil ODS-5). The mobile phase A consisted of 5 mM ammonium formate, water and 0.1% formic acid. The mobile phase B consist of 5mM ammonium formate 0.1% formic acid and methanol. The injection volume of sample was 4µL. HPLC was run at 0.5mL/min flow. The multiple reaction monitoring (MRM) mode was used to quantify the analyzes. The optimum ESI conditions were set as 350° C for interface temperature, 250° C for DL temperature and 400° C for heat block temperature, 3L/min for nebulizing gas flow and 15L/min for drying gas flow. The analyses of samples were carried out after three transitions for the samples. First transition was for quantitative aim and second and third transitions were for verification.

**RESULTS AND DISCUSSION**

**Result of LC-MS Analysis of Ethanol Extract of C. Crepioides**

![Table](image)

Figure 1: m/z ratio of compounds identified against Leaves extracts of *C. Crepioides*
Figure 2: Spectra for C. crepioides

Table 1: Compounds identified from Figure 1

<table>
<thead>
<tr>
<th>S/N</th>
<th>Identified compounds</th>
<th>Molecular formula</th>
<th>Calculated mass</th>
<th>Precursor ion, m/z</th>
<th>Fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sinapic acid</td>
<td>C_{11}H_{12}O_{5}</td>
<td>225.402</td>
<td>224.212</td>
<td>164, 149, 208, 164, 193, 179</td>
</tr>
<tr>
<td>2</td>
<td>3-Feruloylquinic acid</td>
<td>C_{17}H_{20}O_{9}</td>
<td>367.511</td>
<td>367.1034</td>
<td>298, 288, 192, 191</td>
</tr>
<tr>
<td>3</td>
<td>Dihydroquercetin</td>
<td>C_{15}H_{10}O_{7}</td>
<td>303.877</td>
<td>303.0508</td>
<td>285; 163; 267; 159; 239</td>
</tr>
<tr>
<td>4</td>
<td>Malic acid</td>
<td>C_{4}H_{6}O_{5}</td>
<td>134.122</td>
<td>133</td>
<td>115</td>
</tr>
<tr>
<td>4</td>
<td>Malic acid</td>
<td>C_{4}H_{6}O_{5}</td>
<td>134.121</td>
<td>133</td>
<td>115</td>
</tr>
<tr>
<td>5</td>
<td>Hexose-hexose-Nacetyl</td>
<td>C_{14}H_{25}N_{10}O_{10}</td>
<td>365.177</td>
<td>366</td>
<td>186; 142</td>
</tr>
<tr>
<td>6</td>
<td>Gallic acid</td>
<td>C_{7}H_{6}O_{5}</td>
<td>172.152</td>
<td>171</td>
<td>126</td>
</tr>
</tbody>
</table>

Biological Activity of the Leaves extracts of C. crepioides

Table 2: α-amylase activity of the Leaves extracts of C. crepioides

<table>
<thead>
<tr>
<th>DOSE</th>
<th>C. crepioides</th>
<th>ACARBOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>75.8169935</td>
<td>42.4836601</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>76.4705882</td>
<td>58.0065359</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>77.2875817</td>
<td>63.8888889</td>
</tr>
<tr>
<td>250 µg/ml</td>
<td>77.9411765</td>
<td>67.1568627</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>81.6993464</td>
<td>68.627451</td>
</tr>
</tbody>
</table>

Table 2 shows that the inhibition activity of both plants is dependent on the dosage. The leaves extracts of C. crepioides showed decrease in activity with increase in dosage (500 µg/ml). At 250 µg/ml dose the activity of C. crepioides was found to be better than that of the antidiabetic drug acarbose.

Table 3: DPPH Activity of Leaves extract of C. crepioides

<table>
<thead>
<tr>
<th>DOSE</th>
<th>C. crepioides</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>24.71812</td>
<td>30.07525</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>42.57524</td>
<td>41.82336</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>70.67672</td>
<td>51.97373</td>
</tr>
<tr>
<td>250 µg/ml</td>
<td>75.65792</td>
<td>60.80831</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>76.12784</td>
<td>65.41357</td>
</tr>
</tbody>
</table>

Table 3 above shows the DPPH inhibition assay of C. crepioides. The activity of the extract is dose dependent. The activity of C. crepioides decrease with corresponding increase in the concentration of the extract but showed the best activity at minimum dosage. The activity of C. crepioides is better than that of the control at all dosages.
Table 4: H<sub>2</sub>O<sub>2</sub> activity for Leaves extract of C. crepioides

<table>
<thead>
<tr>
<th>Dose</th>
<th>C. crepioides</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>5.1269</td>
<td>11.8782</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>27.6311</td>
<td>26.6836</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>63.0457</td>
<td>39.4755</td>
</tr>
<tr>
<td>250 µg/ml</td>
<td>69.3232</td>
<td>63.2826</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>69.9154</td>
<td>74.6193</td>
</tr>
</tbody>
</table>

Table 4 above shows the H<sub>2</sub>O<sub>2</sub> activity of C. crepioides. The table shows that the inhibition activity of C. crepioides leaves extract is dose-dependent. There is increase in the activity of both polyphenolic rich extract with decrease in dosage with optimum activity achieved at dosage of 20 µg/ml. At this dose the activity was found to be better than that of the conventional positive control ascorbic acid.

Table 5: ABTS inhibition assay of the Leaves extracts of C. crespioides

<table>
<thead>
<tr>
<th>Dosage</th>
<th>C. Crepioides</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>16.2119</td>
<td>27.39433</td>
</tr>
<tr>
<td>50 µg/ml</td>
<td>21.1878</td>
<td>55.80524</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>75.70894</td>
<td>78.22365</td>
</tr>
<tr>
<td>250 µg/ml</td>
<td>90.10166</td>
<td>86.35634</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>91.97432</td>
<td>90.10166</td>
</tr>
</tbody>
</table>

Table 5 shows the activity of the leaves extract of C. crespioides on ABTS inhibition assay. The result showed that the activity of the extract is dose dependent as the activity decreases with corresponding increase in the dosage. The best inhibition on ABTS (16.21) was recorded at 20 µg/ml which was better than that of the control.

Discussion

In this current study, a total of six (6) compounds were tentatively identified by comparing the mass to charge m/z of the compounds as perceived by the LC-MS spectrometer and the structures elucidated above. The classes which these compounds fall include Hydroxycinnamic acid: compounds that fall including this category is 3-feruloyl quinic acid (2) and sinapic acid (1) Hydroxybenzoic acid which includes gallic acid (6) Flavones: Dihydroquercetin (3). Other compounds include malic acid (4).

Hydroxycinnamnic Acid

Result interpreted from the LC-MS spectrum shows that 3-feruloyl quinic acid (2) and sinapic acid (1) fall in the class of hydroxycinnamnic compounds or other phenolic acid derivatives. Compound (2) was identified as 3-feruloyl quinic acid precursor ([M − H]− m/z at 367.1038) which was found to be present in both leaves of Crassocephalum crepioides confirmed by the fragments at m/z 298, m/z 288, m/z 192 and m/z 191, corresponding to the loss of [M-H-3H<sub>2</sub>O-CH<sub>3</sub>], [M-H-H<sub>2</sub>O-CH<sub>3</sub>-HCOOH], [M-H-C<sub>7</sub>H<sub>13</sub>O<sub>5</sub>] and [M-H-C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>], respectively (Wang et al., 2017).

Compound (1) characterized as Sinapic acid with precursor ([M − H]− m/z at 225) which was found present in the extract of Crassocephalum Crepioides and confirmed by fragments at m/z 179, m/z 153, and m/z 210 which was corresponded to the loss or cleavage of [M-H- HCOOH], [M-H-CH<sub>2</sub>CHCOOH] and [M-H-CH<sub>3</sub>] respectively (Lin et al., 2019).

Hydroxycinnamic acids (HCAs) are important phytochemicals possessing significant biological properties. HCAs are widely distributed across the plant kingdom and are plentiful in whole grains, tea, coffee, red wine, numerous fruits, and vegetables (Sroka and Cisowski, 2003). They have been classified as both bioactive components of food and structural and functional components of plant cell walls. HCAs have a basic phenylpropanoid structure as...
as their chemical core. Since quinic acid or the glucose molecule serve as their basic building blocks, natural HCAs often occur as either free forms or esters (Kim et al., 2006). In plants, however, they can also take on more complex derivatives including dimer, trimer, or mixed glycosidic forms. The most noteworthy HCAs are para-coumaric acid, ferulic acid, sinapic acid, and caffeine. Their derivatives have a wide range of biological actions, including antitumoral, antibacterial, antioxidant, and neuroprotective properties.

The phytochemical sinapic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) is widely present in spices, citrus and berry fruits, vegetables, cereals, and oilseed crops. It is known to have antibacterial, anti-cancer, anti-inflammatory, anti-mutagenic, antioxidant, and anticancer properties (Chunye, 2015). The ability of sinapic acid to neutralize the paramagnetic stable radical of 2,2-diphenyl-1-picrylhydrazyl is another property that it is known to possess (DPPH). Sinapic acid inhibits DPPH by 33.2% (Sawa et al., 1998), 88.4% (Nenadis and Tsimidou, 2002), and 50% at concentrations of 0.02 mM, 0.5 mM, and 0.3 mM, respectively, according to the literature. Furthermore, at concentrations greater than 200 M, the 8-bislactone-dimer of sinapic acid exhibits DPPH scavenging action. These amongst other points could be the reason for the good antioxidant property of the fruit as proposed by ethnomedicinal uses of the plant.

**Hydroxybenzoic Acids**

One compound is found in this group which is Gallic acid (6) with a m/z of 171 with a fragmentation of m/z 126 due to loss of [M-H= CO₂]. Gallic acid is a plant-derived phenolic acid, or bioactive molecule. It contains antioxidant effects and may provide further health advantages. According to some research, gallic acid lowers excessive fat storage in obese people by decreasing lipogenesis. Lipogenesis is the process by which fat is synthesized within the body from substances such as carbohydrates. Gallic acids provides efficient protection against oxidative damage caused by reactive species often encountered in biological systems including, hydroxyl (HO•), superoxide (O₂•−), and peroxyl (ROO•−) and the non-radicals, hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCI). Furthermore, GA has been demonstrated as the chief antioxidant component responsible for the efficient antiradical and anticancer properties of a number of plant extracts. Similarly, gallic acid derivatives (GADs) have also been found in a number of phytomedicines with diverse biological and pharmacological activities, such as ROS scavenging, interfering the cell signaling pathways, and apoptosis of cancer cells.

**Flavones**

Compound (5) was identified as dihydroquercetin [M − H]− m/z at 303.0510 based on the fragment peaks at m/z 285 [M-H-H₂O], m/z 275 [M-H-CO] and m/z 151 [M-H-RDA cleavage] (Chen et al., 2016).

**Other Compounds**

Other compounds identified to be present in the plant extracts include, Malic acid (4). Malic acid, a component of compound (3) with a precursor at [M-H]- m/z 134, is discovered to be present in both the leaves and fruit extract employed in this study. Molecular loss caused the fragment peak at m/z 115 [M-H-H₂O] to appear. According to earlier research, malic acid has a sour, acidic taste. This aids in removing dead skin cells when applied to the skin. Its sourness also stimulates salivation in people who have dry mouths. Malic acid also takes part in the Krebs cycle. To create energy, the body goes through this procedure. A dry mouth is frequently treated with malic acid. It is also used to treat several other conditions, including fibromyalgia, fatigue, wrinkles, and acne (WebMD, 2022).
Biological activity

Salihu and Olabiyi (2017) reported on the antioxidative potential of phenolic compounds in protecting the human body system from free radicals. The extract's phenolics had the ability to remove free radicals, chelate metallic catalysts, activate antioxidant enzymes, reduce alpha tocopherol radicals, and block oxidases (Amic, Davidoavic, Beslo, & Trinajstic, 2003). They may also improve food quality by changing taste, fragrance, color, and flavor (Memmune et al., 2009). The presence of flavonoids and phenolics (gallic acid, chlorogenic, caffeic acid, rutin, quercetin and kaempferol) in the extract may also contribute to lowering cellular oxidative stress and inhibit α-amylase, α-glucosidase, acetylcholinesterase and butrylcholinesterase activities (Adefegha & Oboh, 2015). Singh et al. (2012) reported that hydroxycinnamic acids and their derivatives have significant impacts on blood sugar levels. It may provide therapeutic benefits for people with diabetes. This finding corroborates the result from this study where hydroxycinnamic acids were found to be present in both polyphenolic extracts of the two plants employed giving rise to the good antioxidant activity in the alpha-amylose assay.

Singh et al. (2012) reported that hydroxycinnamic acids and their derivatives have significant impacts on blood sugar levels. It may provide therapeutic benefits for people with diabetes. This finding corroborates the result from this study where hydroxycinnamic acids were found to be present in both polyphenolic extracts of the two plants employed giving rise to the good antioxidant activity in the alpha-amylose and alpha-glucosidase assays. Another biologically useful class of compounds in plant extracts is hydroxybenzoic acids. The inhibitory action of 2-Hydroxybenzonic acid (4-Chloro benzylidene)-Hydrazide which is a derivative of hydroxybenzoic acid was found to remarkably reduce the activity of enzyme as an acarbose. Comparable results were reported, greater orders of -amylase inhibitory effects were seen. The aforementioned findings, however, indicate that the synthetically produced 2-hydroxy benzoic acid benzylidene hydrazide-based Schiff base derivative may be more effective anti-diabetic particles at inhibiting carbohydrate-digesting enzymes and may be a useful strategy in the treatment of diabetes (Anusuya et al., 2020). This assertion explains the increasing trend in the activity of Solanecio Biafrae Fruit extract in this study.

CONCLUSION

The antioxidant, antidiabetic and LC-MS identification of compounds profiling in the leaves extract of Cassoschelum crepioides was carried out in this study. Six (6) compounds of different chemical classes and of diverse biological application were identified in the plant extract base on their m/z ratio. The compounds classes are hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and other benzoic acids. From the biological activity of the extracts, C. crepioides exhibited the good antidiabetic activity which increased with corresponding increase in the dosage of the extract. The best activity was achieved at 250 μg/ml (16.9934641) in the ABTS assay which was far better than that of the control drug acarbose at the same dosage. Similarly, in the antioxidant assay carried out in this study, C. crepioides also exhibited the best activity across all assay when compared with the control drug ascorbic acid.

REFERENCES


Antonia Mourtizkoua, Maria Alepakiya, Marilena Stamouli., Abraham Pouliakisa, Anastasios Skirisa, Petros KarakitsosbEvaluation of serum levels of IL-6, TNF-α, IL-10, IL-2 and IL-4 in patients with chronic hepatitis. Immunology Vol. 33. Núm. 2. April - June 2014;33:41-50 - DOI: 10.1016/j.immuni.2014.01.001


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