Ethanol and Ethyl Acetate Crude Extracts of Corn Silk and Their Antidiabetic Effect

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Abstract: The relevance of herbs in the management of various ailments such as diabetes mellitus have been on the forefront as a result of various side effects associated with the use of synthetic drugs. Plants extracts have been continuously explored as an alternative remedy for treatment of ailments. This has prompted this work to examine the ethanol and ethyl acetate crude extracts of corn silk (Zea mays L) for in-vitro antidiabetic activities. Solvents extraction was performed and the crude extracts were obtained. The ethanol and ethyl acetate extracts were subjected into biological assays and characterization of the extracts was done using Gas-Chromatography Mass spectrophotometer. Identified compounds were screened for toxicity using Online Osiris Server. From the obtained results, IC_{50} of ethanol and ethyl acetate crude extracts against α-glucosidase enzyme was reassuring, also there were providential potentials against β-glucosidase and glucoamylose, moreover, the aldose reductase results of the extracts were promising than the reference drug. The promising activities were due to some of the identified compounds which had earlier being confirmed to possess various antidiabetic efficacies such as Azetidin and Thiazolidine with no toxicity properties. The propitious activity of crude extracts of corn silk was an indicating tool for the continuous search for anti-diabetic remedy.

Key words: In-vitro Antidiabetic Activities • Online Osiris Server • Gas-Chromatography Mass Spectrophotometer • IC_{50}, Toxicity

INTRODUCTION

The uses of medicinal plants to manage and treat ailments associated with humans have been in existence since creation and many pharmaceutical agents have been isolated from plants. Plants have well being examined and found to possess various therapeutic benefits in the management of diabetic mellitus (DM), both type 1 and 2[1]. DM has been defined as a metabolic disease describe by hyperglycemia resulting from insulin secretion defects [1]. Moreover, type 1 is reported to be caused by a lack of insulin secretion from β-pancreatic cells and type 2 being associated with obesity [2]. The geometric increase in the Diabetes mellitus is becoming alarming and its complication has grown into a public health problem [3]. Synthetic drugs are continually produced to manage and treat DM such as glucosidase inhibitor, biguanides, insulin sensitizer and sulfonyl ureas and its complications [4], however, there are side effects associated with the use of these commercially produced drugs such kidney and liver failure, diarrhoea [5, 6]. Consequently, natural products from plants with little or no much pronounced side effects are being considered regularly as alternative to commercially produced drugs and being acceptable for health care by world health organization [7, 8].

Among plants with antidiabetic activities is corn silk (Zea mays L). Zea may L. are popular grown crop, widely cultivated and useful for both human consumption and also has industrial uses such as feed mill for livestock. The silk of the corn is considered to be a waste from the corn plant and widely available worldwide [9]. Reports have it that the corn silk possesses hypoglycaemic, anti-tumor, antioxidant, anti-fatigue and
anti-fungal properties [10]. Flavonoid secondary metabolite had been previously isolated from the corn silk [11] and found to have various biological activities which is not limited to anti-oxidant, anti-aging, diuretic and anti-proliferative activity on human cancer cell lines [10]. It is has been reported that lipid metabolism and oxidative stress play significant role in DM besides hyperglycemia, therefore, it is expedient to engage drugs with many pharmacological properties for the effective treatment of DM [12]. Further search for new bioactive compounds for the management of DM from corn silk (Zea may L.) continues.

The aim of the study was to examine the ethanol and ethyl acetate extracts of the corn silk for anti diabetic efficacies via in-vitro studies.

### MATERIALS AND METHODS

#### Plant Source and Preparation: Zea may L. were harvested in a farm in Ipinsa, Akure south Local Government area, Ondo state, Nigeria on the 10th of May, 2017 and the corn silk removed and dried. The dried samples were powdered using a laboratory scale grinder (Sumeet CM/L 2128945) and sifted through 300µm sieve to obtain the corn silk powder. The powdered sample was quarantined by the federal Ministry of Agriculture, MOORE plantation in Ibadan, Oyo state, Nigeria in 29th May, 2017. The powdered sample was sealed and packed in air tight containers [13].

#### Materials: The materials which include the following α-glucosidase, substrate p-nitrophenyla-D-glucopyranoside (pNPG), β-glucosidase used for the analysis were purchased from sigma Aldrich and funded by TWAS/CIIT postdoctoral research fellowship.

#### Methods: The analysis was done in CIIT, Abbottabad, Pakistan in July, 2017 at Department of Pharmacy, centre, Advanced Drug Research (CADR)

#### Crude Extract Preparation: The powdered sample of 50 g were soaked in 200 ml of ethanol and ethyl acetate differently for five days and later filtered using filtered paper (No 1) and the extract was concentrated using rotary evaporator at 35°C.

#### Characterization of the Crude Extract: The analyses of the compounds in the extracts were run on a GC–MS system (Agilent Varian GC: 4800/3000). The volume injected was 0.2 ml and the column flow rate was 1 ml/min. The oven temperature was adjusted from 40°C (hold for 2 min) to 280°C (hold for 10 minutes) at a frequency of 5°C/ minute, the detector was ion trap detector. The GC–MS mass spectrum data were analysed using Mnova 11.0.1 and the database of National Institute Standard and Technology (NIST).

#### Extraction of Enzyme: Lee [14] and Flanagan [15] procedure were followed without modification on the extraction of Maltase glucoamylase.

#### Maltase Glucoamylase Inhibition Assay: This assay was done following the method of Tanaka [16].

#### α-D-Glucosidase Inhibition Assay: This was done by method described by Ma [17].

#### β-Glucosidase Inhibition Study: Was carried out following the recommended procedure of Pérez et al. [18] without modification.

Aldehyde reductase (ALR1) and Aldose reductase (ALR2) inhibitory Assay activities were carried out according to Hayman and Kinoshita [19] without modification.

#### Statistical Analysis: The total percentage inhibitions were calculated [17]. The IC₅₀ values were calculated using non linear curve fitting program PRISM 5.0 (Graph pad, San-Diego, California, USA).

### RESULTS AND DISCUSSION

The use of natural plants for the management of diabetics traditionally has been a major practice by the herbal traditionalists and also many natural bioactive ingredients have been explored from medicinal plants in the treatment and management of the ailment. The use of corn silk has been explored as many active compounds have been isolated from the plant. The results of the ethanol and ethyl acetate crude extracts and the identification of bioactive components have given providence to the use of the plant in the management of diabetic mellitus. From Table 1, the IC₅₀ of α-glucosidase of both ethyl acetate (33.20±0.41 µg/mL) and ethanol (38.10±0.22 µg/mL) extracts were better than the standard acarbose (234.6±2.01µM), also, the IC₅₀ of glucoamylase of the ethyl acetate (2.60±0.01 µg/mL) and ethanol...
Table 1: The inhibition potentials of the enzymes against the extracts and compared to the standards

<table>
<thead>
<tr>
<th>Extracts</th>
<th>α-glucosidase IC50±SEM (µg/mL)</th>
<th>Glucoamylase IC50±SEM (µg/mL)</th>
<th>β-glucosidase % inhibition ±SEM</th>
<th>ALR2 IC50±SEM (µg/mL)</th>
<th>ALR1 IC50±SEM (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>33.20±0.41</td>
<td>2.60±0.01</td>
<td>11.10±1.20</td>
<td>1.30±0.21</td>
<td>0.46±0.30</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>38.10±0.22</td>
<td>4.70±0.4</td>
<td>15.20±0.90</td>
<td>0.65±0.20</td>
<td>0.32±0.07</td>
</tr>
<tr>
<td>Acarbosea</td>
<td>234.6±2.01(µM)</td>
<td>234.6±2.01(µM)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Castanospermineb</td>
<td>NT</td>
<td>NT</td>
<td>59.98%</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Vaproicacidc</td>
<td>NT</td>
<td>NT</td>
<td>57.4±10</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Sorbinild</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>3.10±0.20</td>
</tr>
</tbody>
</table>

SEM = standard mean error

a α-glucosidase standard
b β-glucosidase standard
c ALR1 standard
d ALR2 standard (‘Reported IC50 of 3.42 µM of Sorbinil by Rakowitz et al. [20] and Ali et al. 20’)

Fig. 1: Chromatogram of ethanol crude extract of corn silk (Zea mays)

Fig. 2: Chromatogram of ethyl acetate crude extract of corn silk (Zea mays)

Table 2: Identified compound in ethanol crude extract

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Molecular formula</th>
<th>Retention time (min.)</th>
<th>CAS</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-methyl-2-(2-oxopropyl) Furan</td>
<td>C₆H₈O₂</td>
<td>6.89</td>
<td>87773-62-4</td>
<td>138</td>
</tr>
</tbody>
</table>

Table 3: Identified compounds in ethyl acetate crude extract

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Molecular formula</th>
<th>CAS</th>
<th>Retention time(min)</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazolidine, 2-Ethyl-</td>
<td>C₇H₁₂NS</td>
<td>24050-09-7</td>
<td>3.95</td>
<td>117</td>
</tr>
<tr>
<td>Beta-L-Arabinopyranoside, Methyl</td>
<td>C₁₀H₁₀O₅</td>
<td>1825-00-9</td>
<td>3.84</td>
<td>164</td>
</tr>
<tr>
<td>3-Azetidin-1-Yl-Propionic Acid, Methyl Ester</td>
<td>C₁₀H₁₀O₅N</td>
<td>900188-74-4</td>
<td>3.38</td>
<td>143</td>
</tr>
</tbody>
</table>
(4.70±0.20 µg/mL) extracts were better than the acarbose standard. The β-glucosidase of ethyl acetate (11.10±1.20%) and ethanol (15.20± 0.90%) extracts were more potent than when compared to the standard, castanospermine (59.98%). The IC₅₀ of aldose reductase enzymes (ALR1) of the extracts; ethyl acetate (0.46±0.30µg/mL) and ethanol (0.32±0.20µg/mL) were better than the vaproic acid standard (57.4±10.0%), also the ALR2 of minimum inhibition concentration at 50 (IC₅₀) of the two extracts were better than that of sorbinil standard (3.10±0.20µg/mL). In another development, Figure 1 showed the chromatogram of ethanol extract with only one compound identified at retention time 6.89 minutes and at relative abundance of 100 %. Table 2 revealed the molecular formula, CAS and molecular weight of the identified compound, 3-methyl-2-(2-oxopropyl) Furan. Figure 2 indicated the chromatogram of ethyl acetate of the extract at different peaks and the identified compounds were revealed in Table 3, having different retention times, molecular weight, Vis-a-Vis; Thiazolidine, Arabinopyranoside and azetidin compounds. Table 4 revealed the various drug properties of the identified compounds such as drug likeness and their toxicity. Thiazolidinid compound had no toxicity with +0.58258 drug likeness; also azetidin had 0.19917 drug likeness. The use of plant in treatment of DM had been well enumerated; the potential therapeutic treatment for diabetic conditions using flavonoid extract of corn silk had been reported by Yan Zhang et al. [22]. Moreover, it had been that reported that the action of α-glucosidase (EC.3.2.1.20) catalyzes the final step of carbohydrate digestion in biological systems [23- 27]. This biological importance of α-glucosidase prompts various efforts to develop new agents capable of efficiently inhibiting α-glucosidase [28]. Furan derivatives have been found to possess various pharmacological actions on many ailments including diabetics and found in many plants. Various substituted furans are used as commercialpharmaceutical agents, flavor and fragrance compounds, insecticides and anti-leukemic agents [29], the presence of Furan in the extract may be responsible for the promising activity of the extract on the enzymes. Thiazolidinederivatives possess various antidiabetic activities [30]; moreover, azetidin derivatives have been reported as having a potential anti-inflammatory effect, as well as analgesic and anti-tuberculosis activities [31].

**CONCLUSION**

From the research, it is significant to know that the identified compounds which have been differently found to possess anti diabetic activities may be responsible for the active potential of the corn silk (Zea may L.) against the tested enzymes.

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**Significance Statement:** This study discovered the antidiabetic efficacy of ethanol and ethyl acetate extracts of corn silk, this study will help the researchers to uncover the active bioactive compounds as revealed by the use of gas chromatography mass spectrophotometer. Thus, a new theory on isolating new antidiabetic agents from the corn silk may be arrived at.

**REFERENCES**


