In vitro antioxidant and antidiarrheal activities of aqueous and n-hexane extracts of Cucurbita maxima seed in castor oil-induced diarrheal rats

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ABSTRACT: Diarrhea is a common health complaint occurring with mild, temporary conditions to a potentially life-threatening condition. Cucurbita maxima (Cucurbitaceae) seed is reportedly used traditionally for the treatment of diarrheal and thus this study aimed to evaluate the in vitro antioxidants, total tannins, phenolics contents, and antidiarrheal potentials of n-hexane and aqueous C. maxima seed extracts in castor-oil induced diarrheal rats. The n-hexane extract mainly contains oils while the aqueous extract was thick, brown solid. The aqueous and n-hexane seed extract of C. maxima expressed significant 2,2, diphenyl-1-picrylhydrazyl (DPPH) scavenging activities at 6.25 – 1000 mg/mL with IC_{50} values of 104.01 mg/mL, 29.27 mg/mL and 26.78 mg/mL for n-hexane, aqueous and vitamin C respectively. The hydroxyl radical scavenging activities of the n-hexane and aqueous seed extract of C. maxima were significantly lower at higher concentrations compared with that of vitamin C. Furthermore, the total antioxidant capacities of n-hexane (22.08mg/mL) and aqueous seed extract (11.03 mg/mL) of C. maxima were found to be higher than that of vitamin C (134.46 mg/mL). The aqueous extracts (658.33±380.08 mg QE/g) total tannins were not significantly different from the n-hexane extract (468.33±102.55 mg QE/g) while the n-hexane extract contains significantly higher total phenolics (2.93 mg/mL) for n-hexane, aqueous and vitamin C respectively. The study concludes that aqueous and n-hexane seed extracts of C. maxima (pumpkin) possess in vitro antioxidant activities and antidiarrheal properties.

1. INTRODUCTION

Cucurbita maxima (pumpkin) is a member of the Cucurbitaceae family of plants. In addition to having medical and nutritional benefits, pumpkin is a commercially important vegetable crop (El-Aziz et al., 2011; Kaur et al., 2020). Pumpkins are squash-like fruit that has different size ranges (Orsolek et al., 2000). They are eaten as food and medicinally important (Kaur et al., 2020). All parts pumpkin contain important bioactive components including β-carotene, vitamins (thiamine, riboflavin, K and B6), moderate amount of carbohydrates and minerals (selenium, potassium, phosphorus, magnesium and iron) (Rakejejeva et al., 2011). The seeds are edible with rich protein content and are used as food supplements due to high content of micro and macro minerals such as zinc, phosphorus, manganese, magnesium, copper and calcium (Kaur et al., 2020). Medicinally, pumpkins prevent chronic ailments, possess glucose-lowering activity, antioxidation, antilipogenic effect, anti-inflammation, anticarcinogenic and antiangiogenesis (Caili et al., 2006; Kaur et al., 2020; Wang et al., 2012).

Diarrhea is a common health complaint, with temporary mild condition to a potentially life-threatening condition. Diarrheal is characterized by an abnormal loose or watery stool, which are usually caused by infections with parasites, viruses or bacteria. The conditions are due to imbalanced occurring in the secretory or absorptive physiologies of electrolyte and water (Whyte & Jenkins, 2012). Some pathogenic agents, such as enteropathogenic bacteria, Giardia and cryptosporidium are causes of persistent diarrhea (Shrivastava et al., 2020). The treatments of diarrhea are often done using medications which inhibits prostaglandin secretions, antisecretionary and antispasmodic drugs, such as loperamide, atropine and diareyl (Agbor et al., 2014; Rang et al., 2007). Some of these medications are associated with side effects such as drowsiness, constipation, and colorectal cancer (Rang et al., 2007). In some rural areas, with little access to these medications, plant and plant products are used in management of diarrhea gastrointestinal disorders (Sebai et al., 2014). This study was design to evaluate...
the in vitro antioxidant and antidiarrheal activities of *C. maxima* (pumpkin) seed, which is used in the management of diarrhea.

2. MATERIALS AND METHODS

2.1. Chemicals and Drugs

2,2, diphenyl-1-picrylhydrazyl (DPPH), H₂O₂, n-hexane, ascorbic acid, FeCl₃, H₂SO₄, gallic acid, HCl, glacial acetic acid, sodium phosphate, ammonium molydate were obtained from M&B, England. Castor oil was purchased from El Hawag, Egypt. Loperamide hydrochloride was purchased from Zunamediks, Nigeria. The rest of the chemicals are of analytical quality.

2.2. Plant Material and Preparation

*C. maxima* (pumpkin) seed were purchased from Azare market, Katagum Local Government, Bauchi State, Nigeria and identified at the Department of Biological Sciences, Bauchi State University, Gadau, Nigeria. The pumpkin seed were grounded with a pestle and mortar. The oil was extracted with n-hexane at room temperature by weighing 20 g of the powder seed in 250 mL of n-hexane for 24 hours in an airtight stopped glass container, after which the filtered shaft from the n-hexane extract were dissolve in 250 mL of water for 24 hours. The filtrates were then concentrated separately using a rotary evaporator at 40°C to obtain the n-hexane extract and aqueous extracts respectively.

2.3. *In vitro* Antioxidant Assay

The methods of Mccune and Johns (2002); Smirnoff and Cumbes (1989), and Ruch et al. (1989) were used to evaluate the DPPH, hydroxyl (·OH) and H₂O₂ radical scavenging activities of the extracts respectively. The method of Prieto et al. (1999) was used to evaluate the total antioxidant capacity of the extracts. The methods of Belguidoum et al. (2015), using Folic-ciocalteu was used to determine the total phenolic contents (TPC).

2.4. Animal Care

Adult albino rats were obtained from the animal holding unit, Department of Pharmacology Bauchi State University, Gadau, Nigeria. The animals were housed in standard plastic cages and acclimatized for a period of 2 weeks. They were given unlimited access to animal chaw and water.

2.5. Induction of Diarrheal

Diarrheal was induced in twelve (12) adult albino rats with average weight of 148±10 g by oral administration of 0.5 – 1.0 mL of castor oil after an overnight fasting for 12 hours (Elisha et al., 2013).

2.6. Animal Grouping and Administration for Antidiarrheal Study

The antidiarrheal activities of the n-hexane and aqueous *C. maxima* seed extracts was evaluated by randomly distributing of the animals into 5 groups of 3 animals each. Group one was neither diarrheal induced nor received any treatment. Groups 2 and 3 were diarrheal induced animals, which served as the negative and positive control groups and received 1 mL of castor oil and 3 mg/kg bwt of loperamide respectively. Groups 4 and 5 were diarrheal induced animals, which served as test groups and received 500 mg/kg bwt of the n-hexane and aqueous *C. maxima* seed extracts respectively. All treatment was administered 30 minutes after diarrheal induction and the animals were monitored for up to 4 hours. The faeces (stools) were collected and counted after 2 and 4 hours respectively. The numbers of wet stool passed out were compared with control group.

2.7. Statistical analysis

The data were analysed using one-way ANOVA and the Duncan multiple range test. Data are presented as mean standard deviation (SD). The GraphPad Prism 6 programme was used to create the graphs. The SPSS vs 20, SPSS Inc., Chicago, IL, USA and GraphPad Prism, California, USA were used for the statistical analysis.

3. RESULTS

3.1. Antioxidant Activities of *C. maxima* Seed Extracts

The n-hexane and aqueous seed extract of *C. maxima* expressed significant DPPH scavenging activities for various concentration range of 6.25 – 1000 mg/mL (Table 1). The DPPH IC₅₀ values were 104.01 mg/mL, 29.27 mg/mL and 26.78 mg/mL for n-hexane, aqueous and vitamin C respectively (Figure 1). The hydroxyl radical scavenging activities of the n-hexane and aqueous seed extract of *C. maxima* were significantly lower at higher concentrations compared with that of vitamin C (Figure 2). However, the hydrogen peroxide scavenging activities of aqueous seed extract (50.03 mg/mL) was higher than that of the n-hexane extract (129.20 mg/mL) (Figure 3). Furthermore, the total antioxidant capacities of n-hexane (22.08 mg/mL) and aqueous seed extract (11.03mg/mL) of *C. maxima* were found to be higher than that of vitamin C (134.46 mg/mL) (Figure 4). The aqueous extracts (658.33±380.08 mg QE/g) total tannins were not significantly different from the n-hexane extract (468.33±102.55 mg QE/g) while the n-hexane extract contains significantly higher total phenolics (2.93±1.25 mg GAE/g) compared with the aqueous extract (0.19±0.04 mg GAE/g) (Figure 5 and 6).

3.2. Effects of n-Hexane and Aqueous *C. maxima* Seed Extracts in Castor Oil-Induced Diarrheal Rats

The aqueous extract of the seed of *C. maxima*, castor oil and loperamide treated diarrheal animal showed increased number of stooling (Table 2). The percentage inhibition of the stooling was found to be 37.50%, 33.33% and 100.00% respectively in the experiment for loperamide when compared with the castor oil diarrheal induced without treatment. While the n-hexane and aqueous extract of the seed of *C. maxima* expressed 12.50%, 44.44%, 66.67% and 37.50%, 66.67%, 33.33% respectively.
for 500 mg/kg body weight of the seed compared with the castor oil diarrheal induced without treatment. The average percentage inhibition of stooling was found to be 57±22%, 41±16% and 46±11% for loperamide (3 mg/kg bwt), n-hexane (500 mg/kg bwt) and aqueous (500 mg/kg bwt) respectively.

Figure 1. DPPH radical scavenging activities of n-hexane and aqueous Cucurbita maxima seed extracts. Values are means ± SD of triplicate determinations

Figure 2. Hydroxyl (·OH) radical scavenging activities of n-hexane and aqueous Cucurbita maxima seed extracts. Values are means ± SD Values are means ± SD (n=3)

Figure 3. Hydrogen peroxide (H₂O₂) radical scavenging activities of n-hexane and aqueous Cucurbita maxima seed extracts. Values are means ± SD (n=3)

4. DISCUSSION

Antioxidant characteristics are critical in determining the antioxidant activity of therapeutic plants (Xu et al., 2017). Both ROS and RNS have been implicated in several physiological...
and pathological conditions, and could further involved in disease progression as in diabetic mellitus, atherosclerosis and cardiovascular diseases (Forrester et al., 2018; Liao & Yin, 2000). ROS have been identified as one of the key players in the development of cancer’s various hallmarks. Because ROS are linked to all stages of cancer (Kwon et al., 2019). Recently, Casas et al. (2020) suggested that ROS can be used for numerous key signalling and metabolic processes at any concentration. Nevertheless, ROS remain an important parameter for the evaluation of antioxidants activities (Munteanu & Apetrei, 2021). Imbalance between antioxidant activity and free radical activity (Oxidative stress) is also known to contribute to various gastrointestinal malignancies including gastric and gastrointestinal diseases, including gastroduodenal ulcer (Kekec et al., 2009; Peng et al., 2008). Important in vitro antioxidant assays used in assessing the antioxidant status of medicinal plants include DPPH, hydroxyl radical (•OH), hydrogen peroxide (H₂O₂) and total antioxidant capacity (TAC) as reported in several studies (Munteanu & Apetrei, 2021; Tijjani, Mohammed, Ahmed, et al., 2020; Tijjani, Mohammed, Muktar, et al., 2020). n-hexane and aqueous C. maxima seed extracts demonstrated potent in vitro antioxidant activities, which are comparable with vitamin C in DPPH scavenging and TAC. However, a lower potency was observed in hydroxyl radical and hydrogen peroxide scavenging activities compared with vitamin C. This suggests that the extracts scavenge hydroxyl radicals at a lower capacity. In vivo, H₂O₂ are rapidly decomposed into water and oxygen, preventing the deleterious effects of hydroxyl radical, which can initiate lipid peroxidation and DNA damage (Poljsak et al., 2013; Topal et al., 2015). Plant contains secondary metabolites, which are responsible for several of their biological activities (Tijjani, Adegunloye, et al., 2020; Tijjani et al., 2018). These secondary metabolites are similarly associated with the antioxidant activities of many plants. Flavonoids are for example strong antioxidant compounds (Tijjani et al., 2018). Flavonoids, saponins and triterpenoids are reported to possess the property to inhibit intestinal motility and hydro electrolytic secretions, which are properties altered in diarrheal conditions (Gaginella & Phillips, 1975). In the present study, the higher contents of total phenolic content in hexane extract compared with aqueous extract did not reflect in the in vitro antioxidant status at higher concentration (Figure 1, 2, 3 and 4). The concentration of phenolic may be from the difference in polarity of solvent used in the extraction (Jing et al., 2015). Antidiarrheal medicinal plants are also known to possess antioxidant, antibacterial, and anti-inflammatory properties (Tzung-Hsun et al., 2005).

Diarrhea is a common health problem in most developing countries, especially where there are poor sanitation or hygiene. Diarrhea is the increase in number, fluidity and presence of blood and increased neutrophil polymorphs in the stools (Bern et al., 1992). The model used in the study of antidiarrheal involves the induction of diarrheal in experimental animals using castor oil. The mechanism of induction involves ricinoleic acid, which is the active compound for induction of diarrheal in castor oil. Ricinoleic acid causes inflammation of the intestinal mucosa, leading to increased prostaglandin release, increased peristalsis, and reduced reabsorption of chloride (Cl⁻), potassium (K⁺) and sodium (Na⁺) ions as well as reduced reabsorption of water from the gut, decreased Na⁺,K⁺ ATPase activities in the small intestine and colon, leading to diarrhea (Gaginella & Phillips, 1975; Galvez et al., 1993; Gutiérrez et al., 2014). Other mechanisms reported to involve include activation of adenylate cyclase, mucosal cAMP-mediated active secretion, platelet activating factor and alterations of nitric oxide synthetase pathway (Capasso et al., 1994; Mascolo et al., 1996; Pinto et al., 1992). Diarrheas are treated using drugs like loperamide. Loperamide antidiarrheal activity is linked to inhibition of peristalsis (Fujita et al., 2014; Scarpellini et al., 2016). The present study on the antidiarrheal activities of n-hexane and aqueous C. maxima seed extracts in castor induced diarrheal rats indicated that the extracts from C. maxima seed significantly inhibited the formation of diarrheal stools in castor oil induced diarrheal rats. Their antidiarrheal activities are comparable with the reference drug loperamide. The antidiarrheal activities of the seed oil and extract maybe attributed to protection against gastric irritation, inflammation and reduced prostaglandin release (Ramasamy et al., 2016; Umukoro & Ashorobi, 2005). The antidiarrheal properties may also be link to the rich phytochemical contents of the seed oil and extract (Han et al., 2014; Orshudi et al., 2000; Venkatesan et al., 2005). Flavonoids, for example are reported to inhibit the release of autacoids and prostaglandins, thus inhibiting the motility and secretion induced by castor oil in the rats (Hasan et al., 2009).

Several studies with medicinal plants have also shown that

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; experiment</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; experiment</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; experiment</th>
<th>% of inhibition</th>
<th>Average</th>
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<tr>
<td></td>
<td>No. of stools</td>
<td>No. of stools</td>
<td>No. of stools</td>
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<td>5.00</td>
<td>7.00</td>
<td></td>
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<tr>
<td>Castor oil</td>
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<tr>
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<td>6.00</td>
<td>37.50</td>
<td>33.33</td>
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<tr>
<td>n-HEX (500 mg/kg bwt)</td>
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<td>5.00</td>
<td>2.00</td>
<td>12.50</td>
<td>44.44*</td>
</tr>
<tr>
<td>AQE (500 mg/kg bwt)</td>
<td>5.00</td>
<td>15.00</td>
<td>2.00</td>
<td>37.50</td>
<td>66.67*</td>
</tr>
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n-HEX = n-Hexane extract, AQE = Aqueous extract, mg/kg bwt = mg per kg body weight, Values with * are significantly different at p<0.05 from reference compound.
their antidiarrheal properties are associated with stimulation of water reabsorption, reduced electrolyte secretion, intestinal motility and stimulate antispasmodic effects (Agbor et al., 2004; Kambaska et al., 2006; Lozoya et al., 2002; Oben et al., 2006).

5. CONCLUSION

The study concludes that n-hexane and aqueous C. maxima (pumpkin) seed extracts possess significant antidiarrheal properties. Furthermore, n-hexane and aqueous C. maxima (pumpkin) seed extracts possess significant antidiarrheal properties. Further studies are needed to validate the antidiarrheal activities of C. maxima using charcoal meal intestinal transit and laxative test as well as identifying their active antidiarrheal principles through an activity-guided fractionations and GCMS analysis.

6. CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest associated with this research work.

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ETHICAL APPROVAL

The University of Jos Ethical Review Committee accepted the research with the reference number UJ/FPS/F17-00379.

AUTHOR CONTRIBUTIONS

HT, AIM, MMY, ELA, AS - Research concept and design; HT, AIM, MMY, ELA, AS - Collection and/or assembly of data; HT, AIM, MMY, ELA, AS - Data analysis and interpretation; HT, AIM, MMY, ELA, AS - Writing the article; HT, AIM - Critical revision of the article, HT, AIM, MMY, ELA, AS - Final approval of the article.

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