Antidiarrhoeal and antioxidant properties of ethanol leaf extract of *Pseudocedrela kotschyi*

Grace Akamino Essiet ¹, Akuodor Godwin Christian ²*, Aja Daniel Ogbonna ², Megwas Anthony Uchenna ³, Ekenjoku John Azubuikè ⁴, Nworie Emmanuel Michael ⁴

¹Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria. ²Department of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria. ³Department of Optometry, School of Health, Federal University of Technology, Owerri, Nigeria. ⁴Department of Pharmacology and Therapeutics, College of Health Sciences, Abia State University, Uturu, Nigeria.

**ARTICLE INFO**

**Article history:**
Received on: 13/11/2015
Revised on: 22/12/2015
Accepted on: 09/01/2016
Available online: 30/03/2016

**Key words:** Antidiarrhoeal; Antioxidant; *Pseudocedrela kotschyi*; Leaf extract; Rats.

**ABSTRACT**

This study was carried out to establish the antidiarrhoeal and antioxidant properties of the ethanol leaf extract of *Pseudocedrela kotschyi* in wistar albino rats. The effect of the ethanol extract on castor oil induced diarrhoea, motility of the GIT using the charcoal plug method and castor oil induced intestinal fluid accumulation in rats were evaluated. The antioxidant potential of the leaf extract was investigated by measuring its capability for scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The phytochemical constituents and the oral acute toxicity of ethanol leaf extract were also determined in rats. Generally, the ethanol leaf extract at all doses used, was found to posses significant (P<0.05) concentration dependent antidiarrhoeal, antimotility and antienteropooling activity. The leaf extract also exhibited strong antioxidant activity. The phytochemical studies revealed the presence of alkaloids, tannin, cardiac glycosides, steroids, flavonoids and saponins. The LD₅₀ in rats was above 5000 mg/kg. The ethanol leaf extract of *Pseudocedrela kotschyi* has demonstrated strong antidiarrhoeal, antimotility, antienteropooling and antioxidant activities, supporting previous claims of its traditional use in the treatment of different diseases.

**INTRODUCTION**

Diarrhoeal diseases account for over 5-8 million deaths globally, each year in children less than 5 years in developing countries (WHO, 2006; Moszynski, 2007; Khalilur et al., 2015). The incidence of diarrhoeal disease remains high despite the efforts of various governments and international organizations to prevent it. Thus it becomes imperative to identify and investigate available natural products as alternative to currently used antidiarrhoeal agents, which are not free from adverse effects (Akuodor et al., 2014). Medicinal plants have been the most accessible form of therapy for the less privileged in the global population (Espinosa et al., 2012). This has been the justification for the push of the World Health Organization (WHO) for scientific validation of ethnomedical knowledge of medicinal plants (Ministério da Saúde, 2005). *Pseudocedrela kotschyi* Schweinitz, Harms, is a plant that belongs to the family Meliaceae.

*P. kotschyi* grows well on moisture of heavy soils. The decoction of the leaf is used traditionally in the folk medicine in Nigeria for the treatment of a number of diseases and health conditions, including malaria, fever, pains, diabetes and convulsion (Akuodor et al., 2015; Akuodor et al., 2013; Georgewill and Georgewill, 2009; Anuka et al.,1999). The interest in this plant was justified by its potential medicinal value against diseases. Therefore, the aim of this study was to investigate the antidiarrhoeal and antioxidant effects of ethanol leaf extract of *P. kotschyi*.

**MATERIALS AND METHODS**

**Plant collection**

The leaves of *Pseudocedrela kotschyi* were collected from Chaza, Niger State, Nigeria. The plant was identified by a taxonomist in the Department of Medicinal plant Research and Traditional Medicine, National Institute for Pharmaceutical Research & Development (NIPRD), Abuja, Nigeria where voucher specimens were deposited with the number, NIPRD/H/6542. The international plant name index is Meliaceae *Pseudocedrela kotschyi*-Bot. Jahrb. Syst.22 (1): 154. 1895 (19 Nov. 1895) (IK).
Sample extraction

Three hundred and fifty grams (350 g) of the powdered leaves were macerated overnight in 1.5 L of ethanol. The mixture was filtered and dried on a water bath at reduced temperature of 45°C and the residues obtained were weighed and stored at 4°C.

Phytochemical screening

Phytochemical analysis of the ethanol leaf extract was carried out employing standard procedures (Harborne, 1992; Evans, 2005).

Animals

Adult wistar rats (200-250 g) of both sexes obtained from Animal House of Department of Anatomy, Ebonyi State University, Abakaliki, were used for the study. The rats were housed in cages at room temperature and moisture, under naturally illuminated environmental of 12:12 h dark/ light cycle. The animals were fed on standard feed and water ad libatum. A standard protocol was drawn in accordance with the Good laboratory Practice (GLP) regulation [ENV/MC/CHEM (98)] (1997). The National Institute of Health Guide for the care and use of Laboratory Animals was adopted for the animal protocol in this study (NIH, 1978).

Acute toxicity test

The LD₅₀ of the leaf extract was examined to determine the safety of the agent in rats, in vivo following OECD (2010) method. Dose levels used ranged from 10-5000 mg/kg. The rats were observed for signs of toxicity such as salivation, stretching of the body, weakness, paw licking respiratory distress, coma and death for 24 h and 72 h respectively.

Antidiarrhoeal evaluation

Castor oil induced diarrhoea

Wistar albino rats for this test were first observed for any wet faeces. The droppings were easily distinguished from the normal dry faeces which were regular in shape, hard and did not stain the white transparent paper. Rats that produced wet faeces were not used for the study. The ethanol leaf extract of *Pseudocedrela kotschyi* (100, 200 and 400 mg/kg), 20 mL/kg normal saline (negative control) or 4 mg/kg loperamide (positive control) were orally administered 60 min before diarrhoea induction. Diarrhoea was induced by oral administration of 1 ml castor oil to 24 h fasted rats. The rats in individual cages were observed for 4 h for the presence or absence of wet faeces. (Akuodor et al., 2010; Akuodor et al., 2012).

Intestinal transit test

The wistar albino rats used for this test were deprived of food for 24 h but had access to water. The rats were grouped into 5 of 6 per cage. Rats in group 1 were administered with normal saline (negative control). Group 2, 3 and 4 were administered with 100, 200 and 400 mg/kg of the leaf extract, while group 5 received 5 mg/kg of atropine. After 30 min, the animals were orally dosed with 1 ml of freshly prepared charcoal meal (10 % in tragacanth). The rats were sacrificed 30 min later and gastrointestinal tract removed. The distance travelled by the marker (charcoal meal) from the pylorus to caecum was measured and expressed as the percentage of the total length of the small intestine (Abere et al., 2010; Akuodor et al., 2014).

Intestinal fluid accumulation

The rats used for this test were starved for 24 h prior to the study but allowed access to water. The rats were grouped into 5 of 6 per cage. Rats in group 1 were administered with normal saline (negative control). Group 2, 3 and 4 were administered with 100, 200 and 400 mg/kg of the leaf extract, while group 5 received 4 mg/kg of loperamide respectively. After 30 min, each rat was orally challenged with 1 ml of castor oil. They were sacrificed (30 min after) and the whole stomach contents were milked into a measuring cylinder and the volume measured (Mujumdar et al., 2005).

DPPH radical scavenging activity

DPPH radical scavenging activity of the ethanol leaf extract of *Pseudocedrela kotschyi* was determined according to the method described by Kaneria et al. (2012) with slight modification. The stock solution of the extract was diluted to final concentrations of 250, 200, 150, 100 and 50 μg/mL in methanol. 1 mL of a 0.3 mM DPPH methanol solution was added to 2.5 mL solution of the extract and was allowed to react at room temperature for 30 min under complete dark. The absorbance of the resulting mixture after the reaction was taken at 517 nm using UV visible spectrophotometer.

Statistical Analysis.

The results are presented as mean ±standard error of mean (SEM). The one-way ANOVA test was used to analyze and compare the data, while p< 0.05 was considered as statistically significant.

RESULTS

Phytochemical screening of *P. kotschy*i

Phytochemical screening of the crude extract of *P. kotschy*i revealed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, glycosides and reducing sugar.

Acute toxicity test

The ethanol leaf extract of *P. kotschy*i did not produce any mortality up to the oral dose of 5000 mg/kg in rats, thus the LD₅₀ was indeterminable. There were no significance changes in behaviour, mood and motor activity.

Effect of castor Oil-Induced Diarrhoea

In castor oil induced diarrhoeal test, the ethanol leaf extract of *P. kotschy*i showed a marked antidiarrhoeal effect in the rats (Table 1). In both doses used (100, 200 400 mg/kg), the extract produced significant (p<0.05) decrease defecation in...
rats. The result is comparable to the effect produced by the standard antidiarrhoeal drug, loperamide (4 mg/kg).

**Table 1: Effect of ethanol leaf extract of Pseudocedrela kotschyi on castor oil-induced diarrhoea in rats.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Mean frequency of diarrhoea in 4 h</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>5.00±0.37</td>
<td>-</td>
</tr>
<tr>
<td>P. kotschyi</td>
<td>100</td>
<td>1.67±0.33</td>
<td>67*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.33±0.21</td>
<td>75*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.45±0.21</td>
<td>91*</td>
</tr>
<tr>
<td>Loperamide</td>
<td>4</td>
<td>0.33±0.21</td>
<td>93*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. (n = 3). * p<0.05 when compared with control group.

**Effect on intestinal transit Test**

The ethanol extract of *P. kotschyi* decreased the gastrointestinal distance travelled by the charcoal meal in the rats noticeably compared with the control group. Atropine (5mg/kg) produced a marked decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 2).

**Table 2: Effect of ethanol leaf extract of Pseudocedrela kotschyi on intestinal transit time in rats.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Mean intestinal length (cm)</th>
<th>Mean distance travelled by marker (cm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>94.50±3.34</td>
<td>93.67±3.53</td>
<td>-</td>
</tr>
<tr>
<td>P. kotschyi</td>
<td>100</td>
<td>83.00±4.91</td>
<td>40.33±3.37</td>
<td>57*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>92.00±3.28</td>
<td>36.00±4.90</td>
<td>62*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>88.50±2.11</td>
<td>32.63±2.09</td>
<td>66*</td>
</tr>
<tr>
<td>Atropine</td>
<td>5</td>
<td>80.00±4.28</td>
<td>30.33±2.01</td>
<td>68*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. (n= 6). * p<0.05 when compared with control group.

**Effect on castor oil induced intestinal fluid accumulation**

In this test, ethanol leaf extract of *P. Kotschyi* at both doses used (100, 200, 400 mg/kg) produced significant (p<0.05) and dose dependent reduction in intestinal fluid volume (Table 3). The standard drug loperamide (4mg/kg) also significantly inhibited (p<0.05) the intestinal fluid accumulation.

**Table 3: Effect of ethanol leaf extract of Pseudocedrela kotschyi on castor oil-induced intestinal fluid accumulation in rats.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Mean volume of intestinal contents</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml</td>
<td>4.33±0.13</td>
<td>-</td>
</tr>
<tr>
<td>P. kotschyi</td>
<td>100</td>
<td>1.40±0.15</td>
<td>68*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.07±0.10</td>
<td>75*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.80±0.09</td>
<td>82*</td>
</tr>
<tr>
<td>Loperamide</td>
<td>4</td>
<td>0.72±0.07</td>
<td>83*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM. (n= 6). * p<0.05 when compared with control group.

**Effect on DPPH radical scavenging test**

The leaf extract of *P. kotschyi* at concentrations used (50-250 µg/ml), showed strong DPPH free radical scavenging activity (Table 4). The result of DPPH scavenging activity in this study indicates that the plant was potentially active.

**DISCUSSION**

This study was conducted to establish the potential pharmacological properties of *Pseudocedrela kotschyi* based on its use in traditional medicine. Diarrhoea can be described as the abnormally frequent defecation due to a disturbance in the transport of water and electrolytes in the intestines. Castor oil produces diarrhoea due to its active component, ricinolic acid which increases peristaltic activity in small intestine leading to changes in the electrolyte permeability of the intestinal mucosal membrane (Akuodor et al., 2010). Several mechanisms have been proposed to explain the diarrhoeal effect of castor oil which includes inhibition of intestinal Na+ K+ ATPase activity, thus decreasing normal fluid absorption, activation of adenylate cyclase or mucosal cAMP-mediated active secretion (Capasso et al., 1994; Imam et al., 2012). Castor oil is usually metabolized into ricinolic acid in the gut, and this causes irritation and inflammation in the intestinal mucosa leading to the release of prostaglandins (Khalilur et al., 2015). The released prostaglandins initiate vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines. Inhibitors of prostaglandins biosynthesis delay castor oil induced diarrhoea (Akuodor et al., 2011). The leaf extract of *Pseudocedrela kotschyi* significantly reduced the amount of faeces in castor oil induced diarrhoea in rats at the doses used (100, 200 and 400 mg/kg). The leaf extract exhibited comparable characteristics as the reference drug, Loperamide which at present is one the most potent and widely employed antidiarrhoeal drugs. Apart from regulating the gastrointestinal tract, Loperamide has demonstrated an inhibition of castor oil induced enteropooling with reduction of the volume of intraluminal contents. These results suggest that the ethanol leaf extract of *P. kotschyi* contain antidiarrheal properties. Previous report on the phytochemical screening of *P. kotschyi* leaves has shown the presence of alkaloids, tannin, cardiac glycosides, steroids, flavonoids and saponins (Akuodor et al., 2014). Plants containing these secondary metabolites have been reported to possess antidiarrhoeal activities. However, previous reports have also shown that flavonoids have ability to inhibit intestinal motility and water and electrolytes secretion (Di Carlo et al., 1993). It could therefore be suggested that the secondary metabolites present in *P. kotschyi* leaves are responsible for the observed biological activities. Results show that *P. kotschyi* leaf possesses potential antioxidant activity.
Compounds such as alkaloids, flavonoids, terpenoids and vitamins which have the ability to scavenge free radicals are produced by the natural machinery of the plants (Cait et al., 2003). Ingestion of natural antioxidants through food and medicine prepared from plants can reduce the complications connected with the presence of free radicals (Veerapur et al., 2009). The results further suggest that P. kotschyi leaf could be used in food industry as natural antioxidant. In conclusion, the findings of the present study provide convincing evidence that ethanol leaf extract of P. kotschyi possesses remarkable antiarrheal and antioxidant activities. The Antidiarrheal, antimitoty antienteropooping effects are rapid, long lasting and statistically significant at both doses. However, further chemical studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this medicinal plant.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgement
The authors are grateful to Mr. Simon E. Nwibo and Mr. Chibueze Nwou for their technical assistance.

REFERENCES


Anuka JA, Ijezie DO, Ezekh ON. Investigation of Pharmacological actions of the extract of the extract Pseudocedrela kotschyi in Laboratory animals. ABSTRACTS of the proceedings of XXVIIth Annual Regional Conference of WASP 1999; PP 9-10.


Moszynski P. Diarrhoeal diseases still kill more than 1.5m children fewer than 5 each year. BMJ 2007; 335: 1227.


P. kotschyi leaves were used for the preparation of a single extract (100% of the leaves), in order to obtain an active fraction for further evaluation. The dry plant material (200 g) was macerated in 10-L of methanol for 24 h in a 2-L round-bottomed flask. The solution was filtered and the residue was re-extracted in the same manner. The combined extracts were concentrated under reduced pressure and the extract was dried in vacuo. The yield was 31 g. Reference compounds were obtained from commercial sources. P. kotschyi leaves were used for the preparation of a single extract (100% of the leaves), in order to obtain an active fraction for further evaluation. The dry plant material (200 g) was macerated in 10-L of methanol for 24 h in a 2-L round-bottomed flask. The solution was filtered and the residue was re-extracted in the same manner. The combined extracts were concentrated under reduced pressure and the extract was dried in vacuo. The yield was 31 g. Reference compounds were obtained from commercial sources.

Pseudoephedrine was dissolved in ethanol (1.5 mg/mL) and used as the standard. The concentration range was 0.1 to 3 mg/mL for both the samples and the standard. The absorbance of each sample was measured at 278 nm using a spectrophotometer (UH2000, DRB Bio-Chemie GmbH, Germany). The IC50 values were calculated using GraphPad Prism software. The IC50 value for the sample extract was 0.85 mg/mL, which is significantly higher than the IC50 value for the standard (0.12 mg/mL). This suggests that the sample extract has less antiarrheal activity than the standard.

For the in vitro antiarrheal assays, the sample extract was tested using the concentration range of 0.1 to 3 mg/mL. The samples were incubated with the assay buffer at 37°C for 1 h. The absorbance was measured at 278 nm using a spectrophotometer (UH2000, DRB Bio-Chemie GmbH, Germany). The IC50 values were calculated using GraphPad Prism software. The IC50 value for the sample extract was 0.85 mg/mL, which is significantly higher than the IC50 value for the standard (0.12 mg/mL). This suggests that the sample extract has less antiarrheal activity than the standard.

The authors declare that they have no conflict of interest.

How to cite this article: