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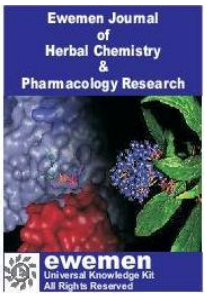

Full Length Research

PHARMACOGNOSTIC EVALUATION AND GASTROINTESTINAL ACTIVITY OF *DRYOPTERIS FILIX-MAS* (L.) SCHOTT (DRYOPTERIDACEAE)

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ABSTRACT

Dryopteris filix-mas have many and varied ethno-medicinal uses, especially its use in the treatment of worm infections and diarrhoea. Hence, this study to evaluate some pharmacognostic parameters and gastrointestinal activity of the methanol leaf extract of *D. filix-mas*. Pharmacognostic evaluation of the leaf was done using standard methods of analysis while the gastrointestinal activity of the extract was evaluated using gastrointestinal transit time, enteropooling and castor oil induced diarrhoea models in rodents. Results of the phytochemical tests showed the presence of glycosides, tannins, flavonoids and steroids in the leaf extract. Quantitative evaluation revealed that Total ash was 9.80%, Acid insoluble ash (0.61%), Water insoluble ash value (1.02%), Moisture content (11.25%), Water extractive value (0.26%), Methanol extractive value (0.82%) and Ethanol extractive value (l.14%). Proximate analysis of the crude drug gave 1.52 % protein, 30.08 % fibre, 2.70 % lipid and 51.46 % carbohydrate. Sodium (200 mg/kg), Potassium (9700 mg/kg), Calcium (6000 mg/kg), Phosphorus (800 mg/kg), Chloride (179.24 mg/kg), Zinc (3.95 mg/kg) and Manganese (0.85 mg/kg) were found to be present. Lead, Chromium, Nickel and Cadmium were not detected. The methanol extract produced a significant ($p = 0.05$) dose - dependent decrease in the gastrointestinal transit time when compared with control (10% Tween 80 solution) and also caused a reduction in intestinal fluid volume. The in-vivo antidiarrhoeal index ($ADI_{in\text{-}vivo}$) of 20.63% produced by the extract (700 mg/kg) was higher than the 16.79% produced by loperamide (2 mg/kg). The results showed that the methanol extract of *D. filix-mas* possess antidiarrhoeal activity, possibly mediated by the reduction of gastrointestinal peristalsis and contains useful phytochemicals which may be responsible for the observed activity. Also, some useful pharmacognostic standards have been established.

Keywords: *Dryopteris filix-mas*, Pharmacognostic standards, Gastrointestinal activity, Rodents**.**

INTRODUCTION

Dryopteris filix-mas (L.) Schott. popularly known as wood fern or male fern belongs to the family Dryopteridaceae. It is an evergreen fern growing to 1.2 m (4 ft) by 1 m (3 ft 3 in) at a medium. It is primarily a specie of moist and fertile forests, particularly deciduous forest, but is also found in a wide range of other habitats including open ground and stone/brick walls in towns (Brandes, 1995), open ground in natural settings (Burga, 1999) and under deciduous scrub in dune hollows (Willis *et al*., 1959).

Biologically, the methanol extract of the leaves of *D. filix-mas* has been found to possess potent antioxidant and cytotoxic activities (Ali *et al*., 2012). The plant also possesses insecticidal activity against *Corcyra cephalonica*, in particular and Lepidoptera in general (Shukla and Tiwari, 2011). It has been found to have antimicrobial activities (Mandal and Mondal, 2011; Soare *et al*., 2012).

Ethnomedicinally, *D. filix-mas* is used for the treatment of worm infections and it is specifically toxic to tape worm (Duke, 1985). It is one of the oldest anthelmintic drug known and has been used since ancient times as worm expellant in both man and animals. Currently, it is the best taeniacidal drug available and is administered in the form of a liquid extract (Mitra, 2006).

In parts of Benin City, herbalists use the alcoholic extract of the plant in the treatment of gastrointestinal ailments such as diarrhoea (Personal communication). Hence this study, which had as its aim, to scientifically evaluate some pharmacognostic parameters and gastrointestinal activity (if any) of the methanol leaf extract of *D. filix-mas*, for its significance in treating diarrhoea and in the balance of both body fluid and electrolytes in the body.

MATERIALS AND METHODS

Materials

All solvents were of the analar grade and were obtained from B.D.H Chemicals, England. Activated charcoal (Ultra carbon charcoal, Merck KGA, Darmstadt, Germany) Loperamide (Loperax®, Xepa Pharmaceuticals, Malaysia), Castor oil (Bell's B Castor oil, Bell Sons and Co. Ltd, England) and Spectrophotometer (Bulk Scientific VGP 210, UK) were also used.

Collection of plant material

The fresh leaves of *D. filix-mas* was collected in the month of March 2015 from a forest in Owo, Owo Local Government Area, Ondo State. Preliminary identification was done by Mr. Sunny Nweke (a plant Curator) of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and authentication was done by Dr. Akinnigbosun of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria. The leaves were air dried for 4 days, transferred to an oven maintained at a temperature of 40°c for 10 minutes and were there after powdered with an electric milling machine. The powdered leaf was stored in an air tight container, labelled and kept until needed for analysis.

Pharmacognostic studies

Phytochemistry

Phytochemical tests were carried out on portions of the powdered leaves of *D. filix-mas* using standard phytochemical procedures (Sofowora, 1982). Phytochemical constituents tested for include glycosides, anthracene derivatives, steroids/triterpenoids, tannins, flavonoids and alkaloids.

Quantitative standards

Quantitative values were established. The parameters determined were; Moisture content, total ash, acid insoluble ash, water soluble ash, alcohol (methanol and 99.8% ethanol) and water soluble extractive values (Shellard, 1958).

Proximate analysis

The nutritive constituents (protein, lipid, fibre and carbohydrate contents) of the powdered plant material were determined using standard methods (Muller and Tobin, 1980; AOAC, 1984).

Elemental analysis

The powdered sample (2 g) was accurately weighed into a clean platinum crucible, ashed at 500ºC, and

cooled to room temperature in a dessicator. The ash was dissolved in 10 mL 20 % nitric acid and filtered into a 100 mL volumetric flask. The crucible was well rinsed with distilled water and transferred to the flask, shaken to mix well and made up to the 100 mL mark with distilled water. Analysis of the sample for elements was carried out in triplicate on a Buck Scientific VGP 210 Atomic Absorption Spectrophotometer (Pearson, 1976).

Pharmacological evaluation

Plant extract

Five hundred grams (500 g) of the powdered leaves of *D. filix-mas* was macerated in 2.5 L of Methanol for 72 hrs after which it was filtered and the filtrate was concentrated over a hot water bath in an evaporating dish to yield 21.63g of extract. The extract was stored in a properly labelled container, adequately covered with foil paper and refrigerated for preservation. At time of use, the extract was made into solution by dissolving it in 10% Tween 80 solution and this was used for the pharmacological tests.

Animals

Fifty Swiss albino mice of both sexes (18 - 32 g) were used for the gastrointestinal and castor oil- induced diarrhoea tests while twenty-five Swiss albino rats were used for the enteropooling (intestinal fluid accumulation) test. The animals were kept in different cages at environmentally controlled room temperature in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. The animals were allowed to acclimatize in the animal house for two weeks and were fed with standard feed. Water was allowed *ad libitum*. They were fasted for 24 hrs prior to the experiment. This research on animals was carried out in the laboratory of Dr. (Mrs.) E. E. Bafor of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City in accordance with the internationally accepted laws governing the use of laboratory animals.

Evaluation of Gastrointestinal activity

Three models were used in the evaluation of the gastrointestinal activity of the methanol extract of the leaf of *D. filix-mas*. The models were: Gastrointestinal transit time in mice, castor oil- induced diarrhoea in mice and enteropooling in rats.

Gastrointestinal transit time model

The method used was according to that described by Hsu (1982). The mice were randomly separated into five groups of five mice each. Group A (control) received 0.2 ml 10% Tween 80 solution; Groups B, C and D received 100, 300 and 700 mg/kg extract respectively while Group E (positive control group) received 2 mg/kg standard solution of loperamide. The administration was done orally using an oro-gastric tube. One hour after the administration, the mice received 0.2 mL each of the standard charcoal meal orally. Thirty minutes after charcoal meal administration, the mice were then humanely sacrificed by cervical dislocation. They were immediately dissected and the small intestine were removed and carefully placed on a clean white surface. The distance travelled by the charcoal meal in relation to the total length of the intestine was measured as the peristaltic index and expressed in percentage for each mouse (Aye-Than *et al*., 1989).

Castor oil- induced diarrhoea

The mice were divided into five different groups of five mice in each group. Group A (control group) received 0.2 ml each of 10% Tween 80 solution. Groups B, C and D received 100, 300 and 700 mg/kg of the extract respectively. Group E (reference group) received 2 mg/kg of loperamide. After an hour of the administration, all the mice received 0.2 ml each of castor oil. Each mouse was placed on a white paper under a transparent cage and observed for four hours (Izzo *et al*., 1992). The following parameters were observed: Onset of diarrhoea, total weight of wet stool, total weight of all stool, percentage protection and in-vivo antidiarrhoeal index.

The in-vivo antidiarrhoeal index ($ADI_{in\text{-}vivo}$) was expressed as:

$$ADI_{in\text{-}vivo} = \sqrt[3]{D_{freq} \times P_{freq}}$$

Where D_{freq} is delay in defaecation time or onset of diarrhoea (in % of control) and P_{freq} is the purging frequency, as amount of stool reduction (in % of control) (Aye-Than *et al*., 1989; Adeyemi and Akindele, 2008).

Enteropooling

The albino rats were separated into five different groups of five rats each. Group A (control) was given 2 ml each of 10% Tween 80 solution. Groups B, C and D received 100, 300, and 700 mg/kg of the extract

respectively. Group E (reference) received 2 mg/kg loperamide. After 30 minutes of administration, 2 ml of castor oil was administered to each of the rats and after one hour, the rats were sacrificed by cervical dislocation, dissected and the intestine was immediately isolated. Each intestine was weighed and the intestinal content drained into a 50 ml beaker. The final volume of fluid was determined using a 2 ml syringe. The empty intestine was then weighed and the difference between the full and empty intestine was determined. The percentage fluid volume was also calculated.

Statistical Analysis

The results were expressed as Mean ± SEM (Standard Error of Mean). The statistical analysis was carried out using One-way analysis of variance (ANOVA) and Turkey's multiple comparison test.

RESULTS AND DISCUSSION

In the pharmacognostic studies, the phytochemical tests carried out on the powered leaves of *D. filix-mas* revealed the presence of carbohydrate, reducing sugar, saponins, tannins, flavonoids and steroids. Anthracene derivatives, Cyanogenetic glycosides and alkaloids were absent. Results of the phytochemical tests are shown in Table 1 below.

Table 1: Results of phytochemical tests for *D. filix-mas* leaf powder

Phytochemicals	Status
Carbohydrates	+
Reducing sugars	+
Anthracene derivatives	-
Cyanogenetic glycoside	-
Saponins	+
Tannins	+
Flavonoids	+
Alkaloid	-
Steroids	+

Key: + means present; – means absent.

Traditional healers and patients in most communities still depend on indigenous herbs for the treatment of diarrhoea, despite the availability of uncomplicated and less expensive diarrhoea treatments such as the oral rehydration solutions (ORS). These herbs are known to contain active phytochemicals which are usually responsible for the use of medicinal plants in the treatment of various ailments. The presence of bioactive compounds such as glycosides, flavonoids, tannins and steroids in *D. filix-mas* leaf powder confirms its use in the treatment of various ailments. Carbohydrates and reducing sugars are organic primary plant metabolites synthesized during photosynthesis. A larger part of the plant is made up of carbohydrates and reducing sugars which are of great pharmacognostic importance, as they combine with a large number of compounds to produce glycosides (Wooton *et al*., 2002). Saponins are glycosides which are made up of a sugar moiety and a non - sugar moiety called "Sapogenins". According to the structure of the sapogenins, saponins can be classified as steroidal and pentacyclic triterpenoids. Flavonoids are a group of polyphenolic compounds known to possess antioxidant properties which include scavenging of free radicals, inhibition of hydrolytic and oxidative enzymes. Hence, flavonoids exhibit biological effects such as antiulcer, antiallergic, anticancer and anti-inflammatory activities. Tannins are phenolic compounds with antimicrobial, antidiarrhoeal, antitumor and anti- HIV activities. Steroids are of great importance in the manufacture of contraceptives and sex hormones (Evans, 2009).The presence of these phytochemicals may be responsible for the observed antidiarrhoeal activity of the methanol extract of *D. filix-mas.*

In the determination of numerical standards, results of quantitative evaluation revealed that Total ash was 9.80%, Acid insoluble ash (0.61%), Water insoluble ash value (1.02%), Moisture content (11.25%), Water extractive value (0.26%), Methanol extractive value (0.82%) and Ethanol extractive value (l.14%). These numerical standards which are identification parameters for medicinal plants were determined in this study as they help guarantee the integrity, genuineness and purity of various medicinal plants or parts of a medicinal plant.

Proximate analysis of the crude drug gave 1.52 % protein, 30.08 % fibre, 2.70 % lipid and 51.46 % carbohydrate. Sodium (200 mg/kg), Potassium (9700 mg/kg), Calcium (6000 mg/kg), Phosphorus (800 mg/kg), Chloride (179.24 mg/kg), Zinc (3.95 mg/kg) and Manganese (0.85 mg/kg) were found to be present. Lead, Chromium, Nickel and Cadmium were not detected. The nutritional (proximate and elemental) qualities of *D. filix-mas* are of great significance, as they could help cater for the decreased absorption of nutrient and increased nutrient requirements which results to loss of weight and lack of growth, especially in children during diarrhoea. They could help replace lost electrolytes while preventing the loss of electrolytes and water.

In the pharmacological tests, results of the gastrointestinal transit time model showed that in the control group, the charcoal meal travelled 98.68% of the total length of the small intestine. The methanol extract of *D. filix-mas* (100 - 700 mg/kg) produced a dose-dependent inhibition, with peak inhibition of 36.24% at 700 mg/kg compared to loperamide (2 mg/kg) with inhibition value of 23.31 % (Table 2).

Table 2: Effect of the methanol extract of *D. filix-mas* on gastrointestinal transit time

Group	Dose (mg/kg)	Peristaltic Index (%)	Percentage Inhibition (%)
A (Control)		98.68 ± 1.32	1.30 ± 1.30
B (Extract)	100	*81.59 ± 4.62	*18.40 ± 4.62
C (Extract)	300	*78.53 ± 3.44	*21.46 ± 3.44
D (Extract)	700	*63.60 ± 10.32	*36.24 ± 10.16
E (Reference)	2	*76.69 ± 8.22	*23.31 ± 8.22

Values are Mean ± S.E.M. n = 5. * p = 0.05, significantly different from control.

Diarrhoea is the frequent passage of watery or loose stools. It involves an increase in the peristalsis of the gastrointestinal tract, with increased excretion of fluid and loss of electrolytes (particularly sodium) and water (Rang *et al*., 2003). It disrupts the body's homeostasis; thus in order to restore normalcy, antidiarrhoeal therapy is required. The therapy is aimed at decreasing peristalsis, hence decreasing the propulsive movement of the gastrointestinal tract and inhibiting the excessive secretion of fluid. It also helps prevent the loss of electrolytes and water (Burks, 1991; Akindele and Adeyemi, 2006). During diarrhoea, decreased absorption of nutrient and increased nutrient requirements combine, resulting to loss of weight and lack of growth, especially in children. The decline in the nutritional status of children results to malnutrition, which in turn worsens diarrhoea (Blossner and de Onis, 2005). Approaches to the treatment of diarrhoea include the use of oral rehydration solutions (ORS), to prevent or treat dehydration and replace lost electrolytes; and the use of antidiarrhoeal medications such as loperamide which is a spasmolytic agent that decreases intestinal motility (NHS, 2014). Others are Kaolin and Pectin, Octretide, Diphenoxylate and Colestipol.

From results obtained in the gastrointestinal transit time model, the extract produced a dose dependent reduction in the propulsive movement of the charcoal meal in the small intestine, with the peak inhibitory effect observed at the dose of 700 mg/kg (36.24%). This inhibitory action on the intestinal transit results in the delay of the passage of the gastrointestinal content, hence increasing the gastrointestinal transit time and decreasing peristaltic movement. The effect causes the stool to become desiccated.

In the castor oil induced diarrhoea model, the methanol extract of *D. filix-mas* produced a better and higher value of the in-vivo antidiarrhoeal index than the reference (loperamide). The onsets of diarrhoea as well as the total weight of stool produced are seen in Table 3.

Table 3: Effect of the methanol extract of *D. filix-mas* on castor oil induced diarrhoea

Group	Dose (mg/kg)	Onset of diarrhoea (min)	Total weight of wet stool (g)	Total weight of all stool (g)	Percentage protection (%)	In-vivo antidiarrhoeal index
A (Control)		56.20 ± 10.23	0.42 ± 0.06	0.42 ± 0.06	0.00	-
B (Extract)	100	84.60 ± 13.86	0.22 ± 0.06	0.22 ± 0.06	52.38 ± 44.72	19.90
C (Extract)	300	71.20 ± 27.53	0.26 ± 0.09	0.26 ± 0.09	61.48 ± 69.77	19.82
D (Extract)	700	88.00 ± 24.30	0.33 ± 0.08	0.33 ± 0.08	78.33 ± 61.56	20.63
E (Reference)	2	101.00 ± 25.45	0.07 ± 0.04	0.11 ± 0.04	26.38 ± 33.17	16.79

Values are Mean ± S.E.M. n = 5.

From the table above, the extract caused a significant delay in the onset of diarrhoea and a reduction in the amount of wet stool produced, when compared with the control. The effect of the methanol extract of the leaf of *D. filix-mas* on all the diarrhoeal indicators as put together by the calculation of the ADI $_{\text{in- vivo}}$ (in-vivo antidiarrhoeal index), showed that the extract produced a dose-dependent increase in ADI $_{\text{in-vivo}}$, with a maximum of 20.63% at the dose of 700mg/kg, when compared with Loperamide (16.79%). The higher the ADI $_{\text{in-vivo}}$ value, the more effective it is in the treatment of diarrhoea.

In the enteropooling model, the methanol extract at a dose of 300 mg/kg caused a 73.55% fluid inhibition unlike the reference (loperamide) which caused a 64.03% inhibition (Table 4).

Thus, there was a reduction in the volume of intestinal fluid expelled in the animals treated with the extract,

with the least volume of fluid expelled at the dose of 700mg/kg of the extract.

Table 4: Effect of the methanol extract of *D. filix-mas* on enteropooling

Group	Dose (mg/kg)	Volume of fluid (ml)	Percentage fluid Inhibition (%)
A (Control)		1.99 ± 0.30	
B (Extract)	100	1.10 ± 0.42	53.31 ± 28.57
C (Extract)	300	1.47 ± 0.90	*73.55 ± 60.43
D (Extract)	700	1.05 ± 0.18	52.40 ± 12.32
E (Reference)	2	1.28 ± 0.19	64.03 ± 13.12

Values are Mean ± S.E.M. n = 5. * p = 0.05, significantly different from control.

CONCLUSION

From the results obtained, this study showed that the methanol extract of *D. filix-mas* (L.) Schott possesses biologically active compounds, which may be responsible for its pharmacological activities. Also, the methanol extract of *D. filix-mas* possesses antidiarrhoeal property through antimotility and antisecretory effects. This thus validates the claim of herbal practitioners, that the plant possesses antidiarrhoeal activity, though safety tests are strongly recommended.

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CONFLICT OF INTEREST

None declared.

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