Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Antimotility effects of extracts and fractions of Eastern Nigeria mistletoe (*Loranthus micranthus* Linn)

Patience O Osadebe, Chika C Abba, Matthias O Agbo*

Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 15 January 2012
Accepted 15 March 2012
Available online 20 July 2012

Keywords:
Loranthus micranthus
Fractination
Gastrointestinal *transit*
Atropine

ABSTRACT

Objective: To evaluate the antimotility activity of Eastern Nigerian mistletoe[*Loranthus micranthus* (*L. micranthus*) Linn] parasitic on six different host trees *viz. Baphia nitida, Persia americana, Kola accuminata, Irvingia gabonensis, Citrus simensis* and *Pentacletra macrophylla* (*P. mycrophylla*). **Methods:** The antimotility of the methanol extracts and solvent fractions were evaluated in castor oil induced diarrheoa in rats. **Results:** The methanol extracts (200 mg/kg, *i.p.*) inhibited defeacation significantly ($P < 0.05$) 4 h after administration (75.73% to 93.33%) more than that of atropine sulphate (2 mg/kg, *i.p.*) which inhibited defeacation by 80.0%. The methanol extract (200 mg/kg, *i.p.*) of *L. micranthus* parasitic on *P. mycrophylla* exhibited significant ($P<0.05$) inhibition in gastrointestinal transit (67.6%) more than that of atropine sulphate (2 mg/kg, *i.p.*) which inhibited gastrointestinal transit by 26.4%. The solvent fractions of *L. micranthus* parasitic on *P. mycrophylla* at dose levels of 150 mg/kg inhibited significantly the gastrointestinal transit of mice. Fraction F_5 exhibited inhibitory activity which was comparable to loperamide (73.3%). **Conclusions:** The methanol extract of *L. micranthus* parasitic on *P. macrophylla* exhibits higher antimotility activity that other extracts. The solvent fractions could serve as source of novel antimotility agents.

1. Introduction

Diarrhoea is a disorder characterized by discharge of semisolid or watery faecal matter from the bowel three or more times in a day[1,2]. It may be acute or chronic. Diarrhoea involves increase in fluidity, volume and frequency of bowel movements, wet stools and abdominal pain, accompanied by increased secretion and decreased adsorption of fluid, and loss of water and electrolyte[3]. Diarrhoea can be very serious in infants and elderly people because of the risk of severe potentially fatal dehydration[4]. Globally, diarrhoea has been estimated to kill about 2.2 million people annually, majority of who are infants and children below the age of 5 years[5,6].

Antimotility agents used in the management of diarrhoea had emerged but none has found a place in the routine management of diarrhoea due to their side effects after prolonged use[7,8]. The search for an alternative remedy among developing countries from traditional herbal medicines was introduced by World Health Organization to overcome the side effects of antimotility agents. Several plants have been reported to be useful in the management of diarrhoea[9-13] .

Loranthus micranthus (*L. micranthus*) Linn (family: Loranthaceae) is a hemiparasitic plant growing on a variety of host trees and shrubs[14]. It depends on its respective host for water and mineral nutrition, even though it produces its own carbohydrates through photosynthesis. It is used in folkloric medicine in the treatment of epilepsy, hypertension, headache, infertility, cancer and rheumatism[15], and has been reported to have antidiabetic, antimicrobial[16], immunomodulatory and antimotility activities[11].

This research is to explore antimotility activity of Eastern Nigerian mistletoe (*L. micranthus* Linn) parasitic on six different host trees.

*Corresponding author: Matthias Agbo, Department of Pharmaceutical & Medicinal Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
Tel: +234-816-266-1991,+234-802-227-7040
E-mail: agbomo2002@yahoo.com;matthias.agbo@um.edu.ngy

2. Material and methods

2.1. Plant material and extraction

Fresh leaves of *L. micranthus* Linn (locally called Owube in Igbo, Kauchin in Hausa and Afomo onisana in Yoruba) parasitic on six different host trees were harvested in the month of May 2008 from Obukpa in Enugu State, Nigeria.

Five hundred gram (500 g) of fresh leaves of *L. micranthus* Linn parasitic on *Baphia nitida, Persia americana, Kola accuminata, Irvingia gabonensis, Citrus sinensis* and *Pentacletra macrophylla* were air-dried at room temperature and pulverized using Thomas-Wiley Laboratory Mill, Model 4. The powdered materials (100 g) were extracted with 500 mL of methanol at room temperature [(20±2)℃] by cold maceration for 24 h. The extraction was repeated with 200 mL of methanol until a clear colour is obtained.

The combined extracts were concentrated to dryness *in vacuo* at (60±2)℃ with a rotary evaporator (Büchi, USA). The percentage yields of the dry extracts were measured.

2.2. Fractionation of Loranthus micranthus extract parasitic on Pentactera macrophylla

The dry extract (10.0 g) was fractionated in a glass column (150 cm × 1.5 cm, ID) packed with 250 g of a slurry of silica gel (60-240 mesh). The column was eluted in succession with 1.5 L *n*-hexane, 1.0 L chloroform, 1.5 L ethyl acetate, 1.5 L acetone and 2.0 L methanol to obtain *n*-hexane, chloroform, ethyl acetate, acetone and methanol fractions (F_1-F_5), respectively.

2.3. Animals

Wistar rats (140-250 g) and albino mice (20-30 g) of either sexes purchased from the animal house of the Department of Pharmacology, University of Nigeria, were used. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light, and allowed free access to standard pelletized feed (Grand Creeds and Oil Mill Nig. Ltd) and water *ad libitum*. The use and care of the laboratory animals were in accordance with internationally accepted best practices as contained in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and approved by the local Ethics Committee of our institution.

2.4. Antidiarrhoea screening of the extracts and fractions

2.4.1. Castor oil-induced diarrhoea in rats

The method of Gerald *et al*[17] modified by Yakubu *et al*[18]; Suleiman *et al*[1], Mbagwu and Adeyemi[2] and Ojewole *et al*[12] was used. Briefly, 48 rats fasted for 18 h (during which the animals were given free access to drinking water *ad libitum*) were randomly allocated to eight groups of six animals each.

Group Ⅰ rats were individually treated with 2 mL/kg (*i.p.*) of 20% tween 80 only. Groups Ⅱ-Ⅶ received intraperitoneally the drug extracts (200 mg/kg, i.p.), respectively dispersed in 20% tween 80. Group Ⅷ was given atropine sulphate (2 mg/kg *i.p.*) in suspension. One hour after intraperitoneally pretreatment of the animals with 20% tween 80, extracts and atropine, each rat in each of the eight groups was given 2 mL/kg of castor oil orally with an orogastric cannula. Each rat was subsequently separately placed in a plastic cage lined at the bottom with a transparent sheet and observed for 4 h for the presence of characteristic droppings. The mean faecal droppings was calculated and expressed as percentage inhibition of diarrhoea using the formula.

$$\text{Percentage inhibition of diarrhoea} = \frac{\text{control mean} - \text{treated mean}}{\text{control mean}} \times 100$$

2.4.2. Gastrointestinal motility in mice

Forty-eight albino mice (20-30 g) of both sexes, fasted for 18 h were randomly divided into eight groups of 6 mice each. Group Ⅰ (control) was administered 20% tween 80 (2 mL/kg, *p.o.*) only while group Ⅱ-Ⅶ individually received 200 mg/kg of *L. micranthus* extracts respectively. Group Ⅷ were treated with atropine sulphate (2 m/kg, *i.p.*). After 60 min of administration of 20% tween 80, extracts, or atropine sulphate, each mouse was given 1 mL of activated charcoal meal (10% charcoal suspension in acacia) orally. After 60 min, animals were sacrificed by anesthesia and the intestine removed without stretching and placed lengthwise on moist filter paper. The length of the intestine (pyloric sphincter to caecum) and the distance travelled by the charcoal as a percentage of that length were evaluated for each mouse and group means were compared and expressed as percentage inhibition[19-21] using the following formula:

$$\%\ \text{Intestine transit} = \frac{\text{Distance travel by charcoal}}{\text{Total lenght of intestine}} \times 100$$

2.4.3. Castor oil induced diarrhoea III

The castor oil induced diarrhoea was repeated in rats using the fractions obtained from Eastern Nigerian mistletoe parasitic on *Pentacletra macrophylla* according to the method described by Agunu *et al*[22], Gerald *et al*[17], Yakubu[18] and Ojewole *et al*[12] with some modifications. Briefly, 42 rats randomly fasted for 18 h but allowed free access to water *ad libitum* was randomly allocated into seven groups of six rats per group.

Group Ⅰ (control) were treated with 20% tween 80 (2 mL/kg,

i.p.), group Ⅱ-Ⅵ were treated with the fractions (150 mg/kg, *i.p.*) suspended in 20% solution of tween 80. Group Ⅶ rats were treated with loperamide (2 mg/kg, *i.p.*). After 60 min, each animal was given 2 mL of castor oil with orogastric cannula, and was placed in a separate cage and observed for 4 h. Transparent sheets were placed beneath each cage and the characteristic diarrhoea droppings were recorded.

2.5. Statistical analysis

Since the data obtained from the antimotility screenings are not normally distributed, the differences between the data obtained from 'test' animal groups and the data obtained from vehicle-treated 'control' animal groups, were subjected to one-way analysis of variance (ANOVA; 95% confidence interval), and followed by Dunnett's *post-hoc* test. In all comparisons, statistical significance was established at values of $P<0.05$.

3. Results

3.1. Preliminary evaluation of antimotility activity

The results of the antimotility activity of methanol extracts of *L. micranthus* parasitic on six host trees are summarized in Table 1.The methanol extracts at dose levels of 200 mg/kg exhibited inhibition in defecation comparable to that of atropine sulphate. The percentage reduction in defecation was the same for the extracts of *L. micranthus* parasitic on *Persia americana*, *Citrus simensis* and *Pentacletra macrophylla*. The methanol extract of *L. micranthus* parasitic on *Irvingia gabonensis* had comparable inhibition in defecation with the standard drug.

This showed that there was variation in the antimotility properties of the extracts due to differences in the secondary metabolities of the host trees. Mistletoe depends on its respective host for water and mineral nutrition, even though it produces its own carbohydrates through photosynthesis.

Table 1
Effects of *L. miconranthus* extract on castor oil induced diarrhoea in rats.

Treatment	Dose (mg/kg, *i.p.*)	Total No. of faecal droppings	Percentage inhibition (%)
Control (20%, 2mL/kg Tween 80)	–	7.50±0.50	–
Baphia nitida	200	1.82±0.52	75.73
Perisia americania	200	0.50±0.29*	93.33
Kola accuminata	200	0.75±0.48	90.00
Irvingia gabonensis	200	1.25±0.63	83.33
Citrus simensis	200	0.50±0.29*	93.33
Pentacletra macrophylla	200	0.50±0.29*	93.33
Atropine sulphate	2.0	3.00±1.08	80.00

One-way ANOVA + Dunnett's *post-hoc* test, $n=6$, *$P<0.05$ *vs*. control.

Table 2
Effects of *L. miconranthus* extract on charcoal meal-stimulated gastrointestinal transit in rats.

Treatment	Dose (mg/kg, *i.p.*)	Mean intestinal length (cm)	Mean distance travelled by charcoal (cm)	Percentage inhibition (%)
Control (20%, 2mL/kg Tween 80)	–	49.13±2.14	49.13±3.08	–
Baphia nitida	200	49.13±2.15	40.83±3.26	16.9
Perisia americania	200	49.13±2.10	42.33±4.09	13.8
Kola accuminata	200	49.13±2.21	25.15±0.86	48.8*
Irvingia gabonensis	200	49.14±2.16	28.96±3.71	41.1*
Citrus simensis	200	48.81±2.15	45.57±5.96	6.6
Pentacletra macrophylla	200	49.14±2.17	15.93±1.09	67.6*
Atropine sulphate	2.0	49.13±2.15	36.14±3.14	26.4*

One-way ANOVA + Dunnett's *post-hoc* test, $n=6$, *$P<0.05$; *vs*. control.

Table 3
Effect of the fractions of mistletoe parasitic on *Pentacletra macrophylla* on castor oil-induced diarrhoea in rats.

Treatment	Dose (mg/kg, *i.p.*)	Total No. of faecal droppings	Inhibition (%)
Control (20%, 2 mL/kg Tween 80)	–	3.75±0.63*	–
F_1	150	1.25±0.63*	66.7
F_2	150	1.50±0.50	60.0
F_3	150	1.75±1.44	53.3
F_4	150	1.50±0.87	60.0
F_5	150	1.00±0.00*	73.3
Loperamide	2.0	1.00±0.00	73.3

One-way ANOVA + Dunnett's *post-hoc* test, $n=6$, *$P<0.05$; *vs*. control.

The effects of the methanol extracts on gastrointestinal transit are shown in Table 2. The extracts from *Kola accuminata* and *Irvingia gabonensis* exhibited higher inhibition of defecation than that of the standard drug (atropine sulphate).

3.2. Antimotility evaluation of the solvent fractions

The results of the effects of the solvent fractions of the methanol extract from *Persia macrophylla* are shown in Table 3. All the fractions at dose level of 150 mg/kg exhibited inhibition in defecation comparable to that of loparamide. The *n*-hexane and methanol fractions exhibited more significant ($P<0.05$) inhibition in defecation than the other fractions. The inhibitions of the gastrointestinal transit of the fractions at a lower dose were comparable to that of the methanol extract of the same host tree. Triterpenoids and alkaloids have been reported to exhibit antimotility properties in experimental animals. The result of the phytochemical analysis of the fractions revealed presence of these secondary metabolities in the fractions.

4. Discussion

The action of castor oil as diarrhoea inductors has been reported[23]. The active component of castor oil is the ricinoleic acid, which produces an irritating activity in the small intestine leading to release of prostaglandins[17,18,24]. This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in hyper-secretory response (decreasing Na^+ absorption), stimulating peristaltic activity and diarrhoea[2]. Clinically, diarrhoea may result from disturbed bowel function, in which case, there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes, and a rapid bowel transit.

The extracts of *L. micranthus* significantly ($P<0.05$) inhibited defecation, at a dose level of 200 mg/kg body weight more than the standard drug, atropine. The prolonged time of induction of diarrhoea, decreased frequencies of stooling and fecal parameters observed with the extracts in this study are indications of antidiarrhoeal potentials of the extracts.

These observations also suggest that the antidiarrhoeal activity of the extracts may be due to the inhibition of prostaglandin biosynthesis. Inhibitors of prostaglandin synthesis and hypersecretion bring about decrease in gastric motility and diarrhoea induced by castor oil.

The extracts of *L. micranthus* parasitic on *Kola accuminata*, *Irvingia gabonensis*, and *Pentacletra macrophylla* inhibited the propulsive movement of the charcoal meal more than the standard drug. The suppressed intestinal propulsive movement of the charcoal meal by the extracts suggests antidiarrhoeal activity of the plant extracts. This may be due to the ability of the extracts to increase the time for absorption of water and electrolytes in the manner similar to the action of atropine sulphate.

The solvent fractions of *L. micranthus* parasitic on *Pentacletra macrophylla* significantly reduced defecation with the methanol fraction (F_5) having the same percentage inhibition in defecation similar to that of loperamide (73.3%). This can be attributed to the polar secondary metabolities present in the fraction. Polar metabolities like alkaloids, flavonoids and saponins were present in the methanol fraction.

The result of phytochemical analysis of the extracts and fractions revealed presence of flavonoids, tannins, terpenoids, steroids, glycoside, alkaloids and saponins. The antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids. While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion[25], tannins denature proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion[26].

The results showed that the extracts and fractions of *L. micranthus* enhanced the re-absorption of water and electrolyte and thus prevents the diarrhoea.

In conclusion, the experimental results obtained from this present study indicate the Eastern Nigerian Mistletoe leaves extracts and fractions sourced from different host trees possess antimotility activity to a varying degree. The difference in the antimotility activities of the extracts is attributed to the relative difference in the secondary metabolites in the host trees. The antimotility activity was high for mistletoe parasitic on *Pentacletra mycrophylla*. These findings authenticate the folkloric claim that *L. micranthus* ethanolic extract or aqueous decoction are used as a traditional remedy for the treatment of diarrhoea.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Mr. A. O. Ozioko of Bioresources Conservation and Development Programme (BCDP), Nsukka.

References

[1] Suleiman MM, Dzenda T, Sani CA. Antidiarrhoea activity of the methanol stem-bark extract of *Annona senegalensis* Pers. *J Ethnopharmacology* 2008; **116**: 125-130.

[2] Mbagwu HOC, Adeyemi OO. Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill. *J Ethnopharmacol* 2008; **116**: 16-20.

[3] Navanecthan U, Giannella RA. Mechanism of infectious diarrhea. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**(11): 637-647.

[4] Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorder. *Gastroenterol* 2006; **130**(5): 1480-1491.

[5] Venkatesan N, Thiyagarajan V, Narayanan, S, Arul A, Raja S, Kumar SGV, et al. Antidiarrhoeal potential of *Asparagius racemosus* wild root extracts in laboratory animals. *J Pharmacol Pharm Sci* 2005; **8**: 39-45.

[6] Gutiẽrrez RMP, Mitchell S, Solis RV. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 2008; **117**: 1-27.

[7] Harrison TR. Diarrhoea and constipation. In: *Principles of internal medicine*. 16th edition. New York: McGraw Hill; 2005, p. 224 -232.

[8] Gale JD. The use of novel promotility and prosecretory agents for the treatment of chronic idiopathic constipation and irritable bowel syndrome with constipation. *Adv Ther* 2009; **26**: 519-530.

[9] Musa AM, Sule MI, Haruna AK, Ilyas M, Iliya I, Yaro AH, et al. Preliminary gastrointestinal studies of methanol extract of *Indigofera pulchra* Willd in rodents. *Nig J Pharm Sci* 2008; **7**(1): 86-92.

[10] Sagar L, Sehgal R, Ojha S. Evaluation of antimobility effect of *Lantana camara L. var*. acuelata constituents on neostigmine induced gastrointestinal transit in mice. *BMC Compl Altern Med* 2005; **5**: 18.

[11] Osadebe PO, Uzochukwu IC. Chromatographic and Anti-motility studies on extract of *Loranthus micranthus*. Linn. *J Pharm Allied Sci* 2006; **3**(1): 263-268.

[12] Ojewole JAO, Awe EO, Chiewororo WDH. Antidiarrhoeal activity of *Psidium guajava* Linn (Myrtaceae) leaf aqueous extract in rodents. *J Smooth Muscle Re* 2008; **44** (6): 195-207.

[13] Osarenmwinda JIP, Omonkhelin JO, Ejiro D. Antidiarrhoeal activity of the methanolic extract of the leaves of *Puallina pinnata* Linn. *Int J Health* 2009; **9**(1): 1-6.

[14] Ali FH, Intesar TN, Khulood WA, Saad AH. Hematopoietic toxicity of *Loranthus europaeus* chloroform extract: *In vitro* study. *Int J Compr Pharm* 2005; **17**: 345-352.

[15] Griggs P. Mistletoe, myth, magic and medicine. *Biochemist* 1991; **13**: 3-4.

[16] Osadebe PO, Ukwueze SE. Comparative study of the antimicrobial and phytochemical properties of Misletoe leaves sourced from six host trees. *J Biolog Res & Biotech* 2004; **2**(1): 18-23.

[17] Gerald NT, Jules RK, Omer BN, Donatien G. Antidiarrhoeal and antimicrobial activities of *Emilia coccinea* (Sims) G. Don extracts. *J Ethnopharmacol* 2007; **112**: 278-283.

[18] Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heirn) stem in male albino rats. *Asian J Androl* 2005; **7**: 399-404.

[19] Rouf R, Uddin SJ, Shilpi JA, Alamgir M. Assessment of antidiarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model. *J Ethnopharmcol* 2007; **109**(3): 539-542.

[20] Mujumdar AM, Misar AV, Upadhye AS. Antidiarrhoeal activity of ethanol extract of the bark of *Dalbergia lanceolaria*. *J Ethnopharmcol* 2005; **102**(2): 213-216.

[21] Aniagu SO, Binda LG, Nwinyi FC, Orisadipe A, Amos S, Wambebe C, et al. Anti-diarrhoea and ulcer-protective effects of the aqueous root extract of *Guiera senegalensis* in rodents. *J Ethnopharmcol* 2005; **97**: 549-554.

[22] Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdurahman EM. Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. *J Ethnopharmcol* 2005; **101**: 27-30.

[23] Jayshree DP, Devang KP, Anshu S, Vipin K. Screening of extracts used in traditional antidiarrhoeal medicines against pathogenic *Escherichia coli*. *Sci World* 2008; **6**(6): 63-67.

[24] Akinde AJ, Adeyemi OO. Evaluation of the antidiarrhoea activity of *Byrsocarpus coccineus*. *J Ethnopharmacol* 2006; **108**: 20-25.

[25] Venkatesan N, Vadivu T, Sathiya N , Arokya A , Sundararajan R, Sengodan G, et al. Anti-diarrhoeal potential of *Asparagus racemosus* wild root extracts in laboratory animals. *J Pharmaceut Sci* 2005; **8**: 39-45.

[26] Brijesh S, Daswani PG, Tetali P, Rojatkar, SR, Antia NH, Birdi TJ. Studies on *Pongamia pinnata* (L.) Pierre leaves. understanding the mechanism(s) of action in infectious diarrhea. *J Zhejiang Univ Sci B* 2006; **7**: 665-674.