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1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, P. M .B. 1017, University of Uyo, Uyo, Nigeria

2. Pharmaceutical Microbiology and Parasitology Unit, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

3. Department of Physiology, Faculty of Basic Medical Sciences, School of Health Sciences, University of Uyo, Uyo, Nigeria

**Correspondence:**
**Dr. Ettebong Ette**
Department of Pharmacology and Toxicology, Faculty of Pharmacy, P. M .B. 1017, University of Uyo, Uyo, Nigeria

# Antiplasmodial and antidiarrhoeal activities of *Dicliptera verticillata* leaf extract

*Ettebong Ette[1]*, Etuk E.U[1], Ubulom Peace[2], Ekpenyong Chris[3], Okokon J.E[1], Udobi C. E[2], Nnadi Eucharia[1], Akpan Idorenyin[1], Akpan Sifonobong[1], Malachy Nsikan[1], Attah Susannah[1]*

## Abstract

*Dicliptera verticillata* (Forssk) Ch. (Acanthaceae) (leaf) is used as a traditional medicine for the treatment of malaria and diarrhoea in Burkina Faso and among the Ibibios of southern Nigeria. This study was aimed at evaluating the antiplasmodial activities of the ethanol leaf extract of *Dicliptera verticillata* in *Plasmodium berghei* infected mice and antidiarrhoeal activity in rats. Suppressive, repository and curative tests were used in determining the antiplasmodial activities in mice following oral administration of the extract (290, 580 and 870 mg/kg). Chloroquine (5 mg/kg) and Pyrimethamine (1.2 mg/kg) were used as standard drugs. The antidiarrhoeal activity of the extract (290, 580, and 870 mg/kg) was evaluated using castor oil-induced diarrhea, fluid accumulation and intestinal transit models. Loperamide (3 mg/kg) and atropine (3 mg/kg) were used as positive controls. The extract showed a dose dependent antiplasmodial activity in the suppressive, repository and curative tests. The mean survival time of the groups treated with extract increased in a dose dependent fashion from 14.33 to 19.33 days compared to control. These results were statically significant ($p<0.001$) compared to the control. Also, there was a dose-dependent reduction in castor oil–induced diarrhoea and this reduction was significant ($P<0.001$). A significant ($P<0.05 – 0.01$) and dose-dependent decrease in intestinal transit and castor oil–induced fluid accumulation was observed. The antiplasmodial and antidiarrhoeal activities of the extract may be due to the presence of alkaloids, saponnins, tannins and flavonoids in the extract. The results of this study confirm the ethnobotanical use of this plant as a malarial and diarrhoeal remedy.

**Keywords:** Antiplasmodial, Antidiarrhoeal, *Dicliptera verticillata*.

## Introduction

*Dicliptera verticillata* is a perennial herb, occurring in the Sahel part of the region from Mauritania to Niger and Northern Nigeria, and distributed throughout tropical Africa, India, Burma and endemic to Christmas Island, Australia, Trinidad and Tobago and Thailand. The plant is also found in Southern Africa, Zambia, Senegal and Sudan and also Lake Chad.[1] Generally, the active principles of *Dicliptera verticillata* include zingiberene, pinene, ascorbic acid, folic acid, riboflavin, carotene, calcium (Ca), Iron (Fe) and Phosphorus (P).[2] This plant has great economic values which have been reported in many countries and continents where it is found. It has been used in traditional medicine for the treatment of fever, diarrhoea, inflammation, malaria, debility, epilepsy and whooping cough[3,4] a remedy for snake bite in Sudan and India, relished for grazing by cattle in Senegal and provides fodder in many other countries such as Zambia, Southern Africa, and Lake Chad.In India, the plant is cut for horse feed, and it is ploughed in as green manure while in southern Africa, a vegetable salt is extracted from it.[5] The decoction of the leaves extracted in water is taken orally to treat anemia in India. The leaves are used in preparation of various soups in India.[6] It is also used as flavouring and spicing in India.[7] The leaves are used in febrile conditions in Ibibio tribe of Akwa Ibom State, Nigeria.[8]

*Dicliptera verticillata* is reported to contain alkaloids, saponins, tannins, phlobatannins, flavonoids, cardiac glycosides and anthraquinones. It possesses antimicrobial, and antidote activities.[5] A yellowish – brown essential oil extracted by steam – distillation was shown to exhibit anti-tuberculostic activity *in-vitro* and inhibits the growth of various strains of *Mycobacterium tuberculosis*. The crude ethanolic extract of the fresh leaves has been shown to have extensive antimicrobial activity.[6,8] The plant has anti-inflammatory, analgesic and antipyretic activities[9] and causes an increase in the Hb concentration and PCV in a dose related manner.[8] However, there is a lack of scientific literature on its antiplasmodial and antidiarrhoeal properties. This present work was embarked upon to ascertain whether the leaf of the plant has any antiplasmodial or antidiarrhoeal potentials as claimed in its ethnomedicinal use. However, further *in vivo* investigations are needed to ascertain and corroborate its activities in humans.

## Materials and Methods

### *Plant materials*

*Dicliptera verticillata* was collected on August 2012 from Itak Ukap, Ikono Local Government Area in Akwa Ibom State. The plant was identified by Dr. (Mrs.) M. E. Bassey, a plant Taxonomist, with Herbarium Number UUH 3094 in the Department of Botany and Ecological Studies, University of Uyo, Uyo.

### *Leaf ethanol extract (LAE)*

The leaves were air-dried under room temperature and powdered using mortar and pestle. The powdered leaves were cold-macerated in an extracting jar, using 70 % ethanol for 72 hours and filtered at room temperature through a Whatman paper filter. The filtrate was then concentrated to dryness *in vacuo* using a rotary evaporator. The dried extract was thereafter stored in the freezer until needed.

### *Phytochemical Screening*

Preliminary phytochemical screening of *Dicliptera Verticillata* for secondary metabolites was carried out using the standard procedure.[10] Screening was carried out to detect the presence of alkaloids, saponins, tannins, phlobatannins, flavonoids, cardiac glycosides, anthraquinones and terpenes.

### *Animals*

Inbred male and female Swiss albino mice (15 – 22 g) and albino rats (90-220 g) were obtained from the animal house of Pharmacology and Toxicology Department, University of Uyo. They were exposed to twelve hours light-dark cycle and were handled according to standard protocol and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum*. Approval for the use of animals in the study was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria with reference number AEC231.

### *Acute Toxicity Study*

An acute toxicity study was carried out to determine the median lethal dose ($LD_{50}$) using the modified method earlier described.[11,12]

### *Malaria Parasites*

A chloroquine- sensitive strain of *P. berghei berghei* (ANKA) was obtained from National Institute of Medical Research (NIMER) in Lagos and maintained by subpassage in mice.

### *Inoculum Preparation*

The parasitized blood donor with high parasitaemia was obtained by first anaesthetizing the mouse with chloroform, and through cardiac puncture blood was collected using sterile syringe into sterile heparinised bottles. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The desired volume of blood, then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculum (0.2 ml) for each mouse contained the required number of parasitized red blood cells (that is $1.0 \times 10^7$ parasitized red blood cells). Therefore, the 0.2 ml of the final inoculum did contain $1x10^7$ parasitized red blood cells, which is the standard inoculum for the infection of a single mouse.[13,14]

### Evaluation of biological activities

### *Evaluation of Antimalarial Activity*

#### *Suppressive Activity (4 day test)*

To determine the suppressive activity of the extract, the methods earlier described with modifications were adopted.[15] This test was used to evaluate the suppressive activity of the extract and chloroquine against early *Plasmodium berghei berghei* infection in mice. On the first day ($D_0$), 30 mice were infected intraperitoneally with 0.2 ml of the infected blood containing the parasite. The animals were then randomly divided into five groups of six mice each. After ten minutes, the drugs were orally administered to the mice. Group 1 served as negative control and received 10 ml/kg/day of distilled water. Groups 2- 4 received 290, 580 and 870 mg/kg/day of the extract respectively. Group 5 received 5 mg/kg/day of chloroquine (positive control). The administration of the extract and drug continued daily for 4 days (Do-$D_3$). On the fifth day ($D_4$), thin blood films were made from tail blood obtained from each mouse and thereafter stained with Leishman's stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope.[16] The percentage parasitaemia was obtained by counting the number of parasitized red blood cells out of 500 erythrocytes in random fields of the microscope.

$$\% \text{ Parasitaemia} = \frac{\text{No. of parasitized RBC}}{\text{Total No. of RBC counted}} \times 100$$

Average percentage chemosuppression was calculated as

$$100 \left( \frac{A - B}{A} \right)$$

Where, A is the average percentage parasitaemia in the negative control group and B, average percentage parasitaemia in the test group.

#### *Repository or Prophylactic Activity*

The methods earlier described with slight modifications were used to assess the prophylactic activity of the extract.[15,17] The prophylactic activity of the extract was assessed by using 36 mice, which were randomly divided into five groups of six mice each. Group 1 served as negative control and received 10 ml/kg/day of distilled water. Groups 2- 4 were administered with 290, 580, and 870 mg/kg/day of the extract respectively, while group 5 was administered with 1.2 mg/kg/day of pyrimethamine (positive control).

All the groups were treated for three consecutive days ($D_o$ –$D_2$) and on day 4 ($D_3$), the mice were intraperitoneally infected with 0.2 ml of infected blood that contained $1 \times 10^7$ *Plasmodium berghei berghei* parasitized red blood cells (RBCs). The level of parasitaemia was assessed by blood smears using thin films obtained from tail blood of each mouse 72 h after the parasite inoculation.Percentage parasitaemia and the average chemosuppression were calculated as stated above.

#### *Curative Activity of Extract*

To assess the schizonticidal activity of the extract during established infection, the method earlier described and modified was used.[18] Parasitized red blood cells containing *Plasmodium berghei berghei* was injected intraperitoneally into 30 mice on the first day ($D_o$). Seventy-two hours later ($D_3$), the mice were randomly divided into 5 groups of six animals per group. Group 1 received 10 ml/kg/day of distilled water and they served as negative control. Different doses of the extract, 290 -870 mg/kg/day was orally administered respectively to mice in groups 2- 4. Group five received 5 mg/kg/day of chloroquine (positive control). All the drugs were administered to the animals once daily for 5 days. Leishman's stained thin smears were prepared from tail blood sample of each mouse collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 30 days ($D_o$ – $D_{29}$).

$$\text{MST} = \frac{\text{Number of days survived} \times 100}{\text{Total number of days (30)}}$$

**Evaluation of Antidiarrhoeal Activity**

***Castor Oil – Induced Diarrhoea***

Albino rats of either sex (90-200 g) were divided into five groups of six animals each. They were fasted for twenty-four hours prior to the tests, but allowed free access to water. Group 1 was treated with 2 ml of distilled water, which served as control. Groups 2, 3 and 4 received different doses of the extract (290, 580 and 870 mg/kg) respectively. Group 5 received standard drug (Loperamide, 3 mg/kg).

All doses were administered orally. The animals were then housed singly in cages. One hour after pre-treatment with the extract, the animals were challenged with 2 ml of castor oil orally. Thereafter, they were observed for five hours in the presence of diarrhoea defined as watery (wet), unformed stool.[19,20]

***Intestinal Transit***

Albino rats of either sex (90 – 200 g) were randomly divided into five groups of six rats each. They were fasted for 24h prior to the test, but were allowed free access to water. The first group was orally administered 2 ml of distilled water. The second, third and fourth groups orally received different doses of the extract (290, 580 and 870 mg/kg) respectively. The fifth group received Atropine orally (3 mg/kg).

Thirty minutes after drug administration, 2 ml of charcoal meal (5% activated charcoal in 10% aqueous tragacanth) was administered to all animals in the study and thirty minutes later, all rats were sacrificed and abdomen opened. The small intestine was dissected out and the distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as a mean distance travelled.[19,20]

***Castor Oil–Induced Fluid Accumulation***

Albino rats of either sex (90-200 g) were divided into five groups of six rats each. They were fasted 24 h prior to the experiment, but allowed free access to water. Group 1 (control) was treated with 2 ml of distilled water. Groups 2, 3 and 4 were treated with different doses of the extract (290, 580 and 870 mg/kg) respectively. Group five was treated with standard drug Loperamide (3 mg/kg). All drugs were administered by oral route. Thirty minutes later, all the rats were challenged with 2 ml castor oil orally. Then thirty minutes later, each rat was sacrificed and the whole length of intestine from the pylorus to the caecum was expelled into a measuring cylinder and the volume measured.[19,20]

**Statistical Analysis**

Results were expressed as multiple comparisons of Mean $\pm$ SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison post- test using Graphpad Instat 3.10 . A probability level of less than 5% was considered significant.

## Results

***Phytochemical Screening***

The results of the phytochemical screening showed that *D. verticillata* contains alkaloids, saponins, tannins, phlobatannins, flavonoids, cardiac glycosides, anthraquinones while terpenes was absent.

***Acute Toxicity Study***

Data obtained from the acute toxicity study were used to calculate the median lethal dose ($LD_{50}$) of the leaf extract of *D. verticillata*. The median lethal dose was calculated to be 2898 $\pm$ 0.10 mg/kg. According to Homburger, this is slightly toxic (500 – 5000 mg/kg).[21] The physical signs of toxicity observed in the experimental mice used in this study were writing, gasping, decreased respiratory rate, and death.

***Suppressive Activity***

The extract showed a dose-dependent suppressive activity on the parasitaemia. These effects were statistically significant relative to control ($p<0.001$). The chemoinhibitory percentages ranged from 59.14 to 83.66 (Table 1). These effects were incomparable with the standard drug chloroquine.

***Repository Activity***

The extract showed a dose dependent repository activity on the parasitaemia. In other words the higher the dose, the percentage reduction in parasitaemia. This effect was statistically significant relative to the control ($p<0.001$). The chemoinhibiting percentages ranged from 59.14 - 94.39 as shown in Table 2.

***Curative Test***

Results obtained from this study reveal that there was a dose-dependent reduction in parasitaemia of extract-treated groups which was significant ($p<0.001$), while the negative control group (distilled water) showed a daily increase in parasitaemia. Chloroquine treated group showed the highest curative effect on parasitaemia at the end of day 7, followed by the high dose, middle dose and lower doses of the extract as summarized in Table 3.

The mean survival time (MST days) of the groups treated with extract increased in a dose dependent fashion compared to the negative control (distilled water) whereas chloroquine gave the highest mean survival time (30 days) as shown in Table 4.

***Castor Oil-Induced Diarrhea***

The ethanolic extract of *D. verticillata* produced marked anti-diarrhoeal effects in the rats, as shown in Table 5. At doses of 290, 580 and 870 mg/kg, the extract caused a significant ($P<0.001$) dose-dependent decrease in the total number of wet feaces produced upon administration of castor oil when compared to the control group. However, the highest dose of the extract, 870 mg/kg produced a level of inhibition (66.09%) that was less than that of the standard anti-diarrhoeal drug, Loperamide (77.99%).

***Intestinal Transit***

The ethanolic extract of *D. verticillata* dose dependently decreased propulsion of charcoal meal in the rat gastro-intestinal tract at oral doses of 290 – 870 mg/kg compared to control as shown in Table 6. The extract showed no significant effect at the lowest dose, 290 mg/kg; but showed significant response at the mid, 580 mg/kg ($P<0.05$) and the highest doses 870 mg/kg ($P<0.001$).The inhibition (36.58%) of the highest dose, 870 mg/kg was slightly lower than that of the standard drug, atropine (42.90%).

***Castor Oil-Induced Fluid Accumulation***

*D. verticillata* was found to possess anti-enteropooling activity. The extract gave a dose-dependent decrease in fluid accumulation which was significant at the highest dose, 870 mg/kg ($P<0.05$) when compared to control as shown in Table 7. However, the inhibition (43.49%) at the highest dose 870 mg/kg was slightly lower than that of the standard drug, Loperamide (52.58%).

**Table 1:** Suppressive activity of extract in mice

| Drug/Extract | Dose mg/kg/day | Parasitaemia | % chemosupression |
|---|---|---|---|
| Distilled water | 10 ml | 69. 33 ± 1.52 | - |
| Extract | 290 | 28.33 ± 2.01* | 59.14 |
| Extract | 580 | 20.33 ± 0.58* | 70.68 |
| Extract | 870 | 11.33 ± 0.92* | 83.66 |
| Chloroquine | 5 | 3.00 ± 0.36* | 95.67 |

Values are expressed as Mean ± SEM
Significance relative to negative control *p< 0.001 (n = 6)

**Table 2:** Repository Activity of Extract in Mice

| Drug/Extract | Dose mg/kg/day | Parasitaemia | % chemosupression |
|---|---|---|---|
| Distilled water | 10 ml | 71.33 ± 0.52 | - |
| Extract | 290 | 32.67 ± 0.84* | 54.20 |
| Extract | 580 | 23.33 ± 0.12* | 67.30 |
| Extract | 870 | 16.00 ± 0.73* | 77.57 |
| Pyrimethamine | 1.2 | 4.00 ± 0.36* | 94.39 |

Values are expressed as Mean ± SEM
Significance relative to negative control *p < 0.001 (n = 6)

**Table 3:** Antiplasmodial activity of Extract during Established Infection (Curative Test) in Mice

| Drug/Extract | Mean ± SEM Parasitaemia | | | | |
|---|---|---|---|---|---|
| | Dose mg/kg | $D_3$ | $D_5$ | $D_6$ | $D_7$ |
| Distilled water | 10 ml | 70.00 + 0.63 | 72.67 ± 0.56 | 78.00 ± 0.35 | 80.33 ± 0.47 |
| Extract | 290 | 71.33 + 0.01 | 25.33± 0.65* | 19.33 ±0.12* | 11.00± 0.97* |
| Extract | 580 | 68.33 + 0.56 | 20.67± 0.56* | 13.67± 0.92* | 7.33 ± 0.21* |
| Extract | 870 | 69.00 + 0.56 | 18.67± 0.53* | 11.00± 0.97* | 3.67 ± 0.56* |
| Chloroquine | 5 | 70.67 ± 0.12 | 11.33± 0.76* | 6.00 ± 0.63* | 2.00 ± 0.36* |

Values are expressed as Mean ± SEM
Significance relative to negative control *p < 0.001 (n = 6)

**Table 4:** The Mean Survival Time (MST: days) of mice receiving various doses of ethanolic leaf extract of *Dicliptera verticillata* during established infection

| Drug/Extract | Dose mg/kg/day | Mean survival time (mst) (days) |
|---|---|---|
| Distilled water | 10 ml | 10.33 ± 0.56 |
| Extract | 290 | 14.33 ± 0.56* |
| Extract | 580 | 16.67 ± 0.21* |
| Extract | 870 | 19.33 ± 0.42* |
| Chloroquine | 5 | 30.00 ± 0.00* |

Values are expressed as Mean ± SEM
Significance relative to negative control *p < 0.001 (n = 6)

**Table 5:** *Effect of D. verticillata Extract on Castor Oil-Induced Diarrhoea in Experimental Rats*

| Group | Dose (mg/kg) | Number of wet faeces 5h | % Inhibition |
|---|---|---|---|
| Control | 10ml | 19.67 ± 0.84 | – |
| Extract | 290 | 8.33 ± 0.92* | 57.65 |
| Extract | 580 | 7.50 ± 0.97* | 61.87 |
| Extract | 870 | 6.67 ± 0.42* | 66.09 |
| Loperamide | 3 | 4.33 ± 0.56* | 77.99 |

Values are expressed as Mean ± SEM. Significance relative control *p<0.001 (n=6)

**Table 6:** Effect of *D. verticillata* Extract on Castor Oil-Induced Intestinal Transit in Experimental Rats

| Group | Dose (mg/kg) | Mean Distance Travelled by Charcoal Meal | % Inhibition |
|---|---|---|---|
| Control | 10ml | 63.7 ± 3.05 | – |
| Extract | 290 | 49.1 ± 2.17 | 22.45 |
| Extract | 580 | 45.7 ± 2.96* | 28.26 |
| Extract | 870 | 40.4 ± 2.27** | 36.58 |
| Atropine | 3 | 36.37 ± 2.26*** | 42.90 |

Values expressed are Mean ± SEM. Significance relative to negative control *p<0.05; **p<0.01; ***p<0.001 (n=6).

**Table 7:** Effect of *D. verticillata* extract on Castor Oil–Induced Fluid Accumulation in Experimental Rats

| Group | Dose (mg/kg) | Volume of Intestinal Content (ml) | % Inhibition |
|---|---|---|---|
| Control | 10ml | 4.07 ± 0.32 | – |
| Extract | 290 | 3.50 ± 0.41 | 14.00 |
| Extract | 580 | 3.17 ± 0.30 | 22.11 |
| Extract | 870 | 2.30 ± 0.40* | 43.49 |
| Loperamide | 3 | 1.93 ± 0.34** | 52.58 |

Values are expressed as Mean ± SEM. Significance relative to control *p<0.05, **p<0.01 (n=6).

## Discussion

In this study, the acute toxicity evaluation of the extract revealed that lethal dose ($LD_{50}$) of the ethanolic leaf extract of *D. verticillata* was only slightly toxic, which shows that this plant is relatively safe.[21]

The phytochemical screening of the leaves of *Dicliptera verticillata* showed the presence of alkaloids, tannins and phlobatannins, saponins cardiac glycosides, flavonoids and anthraquinones. The alkaloids, saponins, tannins and flavonoids have been reported to be responsible for the antimalarial activities of plants.[22] These secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other.[23]

The antiplasmodial results indicate that the leaf extract possessed blood schizontocidal activity as evident from the chemosuppression obtained during the 4 day early infection test, though it could not produce suppression comparable to that of the standard drug Chloroquine. The plant extract also exhibited repository activity, though the dose used could not produce suppression comparable to that of the standard drug pyrimethamine. Chloroquine has a very high volume of distribution, as it diffuses into the body is adipose tissue. Chloroquine is also a lysosomotropic agent, (it accumulates preferentially in the lysosomes of cells in the body). The lysosomotropic character of chloroquine is believed to account for much of its antimalarial activity; the drug concentrates in the acidic food vacuole of the parasite (malaria) and inteferes with the essential processes.[24] Chloroquine enters the red blood cell, inhibiting parasite cell and digestive vacuole by simple diffusion.[25]

The extract (870 mg/kg) was observed to sustain the mice for only for 19 days out of the 30 day period of study, which is lower than that of the standard drug (Chloroquine) thus demonstrating a considerable antimalarial activity. Besides, this lower activity may have resulted from the crude nature of the extract which could be improved by further purification of the extract.

Alkaloids have been known to show antimarial properties by blocking protein synthesis in *Plasmodium falciparum*.[26] Flavonoids have been reported to chelate with nucleic acid base pairing of the parasites.[18] Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial action by

causing elevation of red blood cell oxidation or by inhibiting protein synthesis.[27] The extract could have elicited its action through either of the two mechanisms mentioned above or by some other unknown mechanisms.

Diarrhoea is usually considered as a result of altered motility and fluid accumulation within the intestinal tract.[20] The present study reported the anti-diarrhoeal effect of the ethanolic extract of *Dicliptera verticillata* in various models such as castor oil-induced diarrhoea, gastro-intestinal transit and fluid accumulation in albino rats.

In the present investigation, the ethanolic extract of *D. verticillata* exhibited a significant inhibition of castor oil – induced diarrhoea in a dose-dependent manner. Loperamide is an opioid receptor agonist and acts on the μ-opioid receptor in the mysenteric plexus in the large intestine, but it does not affect the central nervous system like other opioids.[28]

In the evaluation of the gastrointestinal transit atropine sulphate was used as standard drug. Atropine sulphate is known to inhibit gut motility most probably due to its anti-muscarinic effect.[29] The *D. verticillata* extract appeared to act on all parts of the intestine. In this way, they reduced the intestinal propulsion in the charcoal meal-treated model for different transit periods. Activated charcoal prevents the absorption of drugs and chemicals into the system by avidly absorbing them on the surfaces of the charcoal articles.[30] In this study, activated charcoal is used in the gastrointestinal motility test to find out the effects of this extract on the peristaltic movement.

*D. verticillata* extract led to marked reduction in total volume of intestinal contents. This indicates that *D. verticillata* extract reduces diarrhoea by increasing the reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid just as Loperamide.[30] Loperamide acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluid.[30]

The action of castor oil as a diarrhoea inducer is due to its active constituent ricinoleic acid, which is released from the action of lipases in castor oil.[31] This results in irritation to the intestinal mucosa and elicits inflammation, which releases prostaglandins and nitric oxide, responsible to stimulate gastrointestinal secretions, motility, epithelial permeability[32] and edema of the intestinal mucosa, thereby preventing the reabsorption of $Na^+$, $K^+$ and water.[33]

Similarly, *D. verticillata* extract reduced the amount of faecal matter and slowed down the propulsion of charcoal meal through the gastrointestinal tract, and also caused a marked reduction in the volume of intestinal contents. This is often due to increased reabsorption of water, sodium and potassium ion concentration. The sodium and potassium transport in the intestine has been related to membrane bound enzyme sodium and potassium. In diarrhoeal conditions, the decrease in $Na^+$ and $K^+$ ATPase occurs relating to an interception in the normal water and electrolyte absorption. Therefore, the reduction of water together with $Na^+$ accumulation might have an effect on the activity of $Na^+$ and $K^+$ ATPase.[19] Hence, *D. verticillata* extract appears to stimulate the reabsorption of intestinal fluids in the intestine.

Anti-diarrhoeal properties of medicinal plants are known to be due to the presence of tannins, flavonoids, saponins, alkaloids, reducing sugars and triterpenes.[34] The phytochemical studies on *D. verticillata* have revealed the presence of flavonoids, tannin, saponins and alkaloids. Thus, these chemical constituents may be responsible for the in *vivo* anti-diarrhoeal activity of *D. verticillata* extract. Flavonoids have anti-diarrhoeal activity, which have the ability to inhibit intestinal motility and hydroeletrolytic secretions which are known to be altered in diarrhoeal conditions.[35] Flavonoids and alkaloids are known for inhibiting release of autocoids and prostaglandins, thereby inhibiting secretion induced by castor oil.[36,37]

Tannins and tanic acid present in the anti-diarrhoeal plants denature the proteins in intestinal mucosa by forming the protein tannates, which make the intestinal mucosa more resistant to chemical alteration and hence reduce secretion. Tannins act by reducing intracellular calcium inward current or activation of the calcium pumping system which induces the muscle relaxation, thereby reducing peristaltic movement.[38]

### Conflicts of interest

We declare that we have no conflict of interest whatsoever.

## Conclusion

The results of this investigation revealed that *D. verticillata* contains pharmacologically active substances with antiplasmodial and anti-diarrhoeal properties. These attributes may provide the rationale for use of *D. verticillata* in malaria and diarrhoea management by traditional healers. Further research is required to fully investigate the mechanisms responsible for these observed antiplasmodial and antidiarrhoeal activities.

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