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## Research Article

# Effect of Methanolic Extract of Some Selected Plants on the Mortality of Leech (*Hirudo medicinalis*)

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## Abstract

**Background and Objective:** The increased invasiveness of Leeches in many streams, lakes and fishing sites in Bayelsa State has called for a prompt control intervention. This study was undertaken to test the effect of the extract of four botanicals, *Cassia alata*, *Costus afer*, *Ficus sur* and *Platostoma africanum* on the mortality rate of the leech. **Materials and Methods:** The screening, preparation and extraction of the metabolites from the plant samples followed standard techniques. The leeches were collected from a fishing lake and a stream in Elebele Community of Ogbia, Bayelsa State into a sterilized transparent container using a plastic scoop and transported along with their natural habitat water (stream water) to the Department of Biological Sciences' Laboratory. Identification of the leeches followed standard pictorial key. A complete randomized block design was used to set up three treatment groups with varying concentrations of the plant extract. Two hundred and eighty-eight of the leeches previously kept in the aquarium were introduced into the treatments and their death rates were recorded. **Results:** From the results, five phytochemicals, Alkaloids, Flavonoids, Tannins, Terpenes and Saponins were encountered with the plants. Out of the 288 leeches tested with the plant extract, the mortality rate was 61.5%, with more death recorded with *Platostoma africanum*. The mean death rate in *Cassia alata*, *Costus afer* and *Ficus sur* and *Platostoma africanum* ranges from  $11.03 \pm 9.11-100 \pm 0.00$ ,  $22.07 \pm 19.11-100 \pm 0.00$ ,  $11.03 \pm 9.11-100 \pm 0.00$  and  $47.80 \pm 19.22-100 \pm 0.00$ , respectively. **Conclusion:** The mortality rate increases with an increase in extract concentration. The promising anti hirudinicidal activities of these plants should be employed.

**Key words:** Methanolic extract, botanicals, mortality rates, *Hirudo medicinalis*, petechial haemorrhage, blood-borne pathogens, parasite infestation

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Leech (*Hirudo medicinalis*) is a member of the Phylum, Annelida and class Hirudinea. Leeches are widely distributed in different parts of the world<sup>1</sup>. Geographically, over 37 leech species in six families and 19 genera have been described in the North African countries<sup>2</sup> and 13 leech species belonging to two orders, two suborders, four families and 11 genera in Tunisia<sup>3</sup>. Freshwater fish leeches occur in almost all the continents except Antarctica where they are most abundant in the temperate lakes, ponds and streams<sup>4</sup>, while Marine leeches are most abundant in polar to temperate<sup>5</sup>.

Leeches are ectoparasites of fish and amphibians, primarily lamprey, elasmobranch, teleost and frog and toad<sup>2</sup> where they feed on hosts' blood, causes discomfort to the host and transmit several known blood-borne pathogens<sup>6</sup>. The role of leeches in the pathology of fish cultured in the tropics has been studied<sup>7</sup>. Leeches' activities on the host through its attachment may serve as sites for bacteria secondary infection. These activities, usually elicit tissue response in the form of hyperplasia at the attachment sites and initiate a localized petechial hemorrhage<sup>8</sup>. The increased invasion in many lakes and streams in Bayelsa State is a cause for public health concern and have called for a prompt control intervention.

Synthetic chemicals have been used to control different stages of parasites. Reports have shown that these chemicals are Eco toxic and affect non targeted species of organism<sup>9</sup>. The advocacy of environmentally friendly, safe and cost-effective strategies to control diseases pathogens have prompted the use of several plant extracts to control parasite infestation<sup>10,11</sup>. The extracts from *Cassia alata*, *Costus afer*, *Ficus sur* and *Platostoma africanum* have been used to control several ailments<sup>12</sup>. However, the use of these plant extracts in the control of leech is novel in Bayelsa State, Nigeria.

This is therefore a pioneering study aimed at assessing the effect of methanolic extracts of *Cassia alata*, *Costus afer*, *Ficus sur* and *Platostoma africanum* on the mortality of leeches. The result of this study shall provide baseline information on the effectiveness of the methanolic extract of botanicals in the control of leech as an ectoparasite of fish and amphibians.

## MATERIALS AND METHODS

**Description of study location:** Elebele (latitude. 40°1'N and longitude 60°20'E) is one of the ancient communities in the Northeastern part of Bayelsa State in Ogbia Local Government Area. The location is characterized by four seasons<sup>13</sup>, they are

the early rainy season (March-July), little dry season (also called August break), late rainy season (September-November) and dry season (December-February). The mean annual rainfall for the study area is above 3000 mm. The highest rainfall was July (424.6 mm), August (444.6 mm) and September (552.3 mm). The lowest rainfall values were January (42.7 mm) and December (51.1 mm). There are several fishing lakes and rivers in the study location, which are presently waterlogged. This has created enabling environments for the leeches to thrive.

The study was carried out in the Elebele Community of Ogbia, Bayelsa State April 2019-November 2020. The laboratory analysis was done at the Department of Biological Sciences Laboratory, Niger Delta University, Bayelsa State.

**Sources, collection and identification of leeches:** Leeches were collected from a fishing lake and a stream in Elebele Community of Ogbia, Bayelsa State. In each of the sites, along woody stick was used to make disturbances on the water body. These disturbances provoke the leeches into motions. The mobile leeches together with those found attached to substratum along the lake shores were collected into a sterilized transparent container using a plastic scoop. The specimens were transported along with their natural habitat water to the Department of Biological Sciences Laboratory, Niger Delta University, Bayelsa State. The leeches were identified according to the standard method<sup>2</sup>. A total of 288 leeches with lengths ranging from 0.1-4.3 cm were introduced into an improvised aquarium and allowed to be acclimatized for 2 weeks. Three adult catfishes (*Clarias gariepinus*) were introduced into the aquarium as sources of blood meal. The aquarium water was changed periodically for better aeration.

**Sources and collection of plants:** The four plant species, *Cassia alata*, *Costus afar*, *Ficus sur* and *Platostoma africanum* were collected within the Amassoma community. The plants were collected into a clean polyene bag and transported to the Department of Biological Sciences Laboratory, Niger Delta University, Bayelsa State. These plants were identified by an experienced plant taxonomist using standard keys<sup>14</sup> and labelled accordingly.

**Preparation and extraction of phytochemicals:** Before the extraction of phytochemicals, the plant specimens were washed in a distilled water. Thereafter, they were spread in a clean plastic mat where the water in the samples was allowed to drain out. The plant specimens were air-dried at room temperature for 3 days. The air-dried leaves were transferred

into an oven for further drying at 60 until it becomes crispy. The dried leaves were ground to powder using an electronic blender and weighed using an electronic weighing balance.

An equal weight of the grind leaves was introduced into a 250 mL bottle. An aliquot of methanol was introduced into the bottle containing the leaves to soak it. The mixtures were allowed to stand for 72 hrs. After which, the solution was decanted into another 250 mL beaker using 4 folds of fine muslin cloths. The beaker containing the experimental solution was evaporated in a water bath at 40°C to dryness until the extraction was obtained. The extract was stored at 4°C in an airtight bottle for further analysis. This was later dissolved in acetone where different concentrations of the extract were prepared. Preparation of the different concentrations followed standard procedures<sup>9</sup>.

**Experimental design and the biocidal test:** A completely randomized block design technique was used in this study with three treatment groups. Two hundred and eighty-eight of the leeches kept in the aquarium were retrieved. The seven concentrations of each of the four plants extract were replicated. For each concentration, three beakers containing 50 mL of the required concentration of the extracts were set

up. Three leeches were put into each of the replicated beakers and mortality was recorded at 12 hrs using a stopwatch. For control, three leeches were kept in each beaker containing 50 mL of distilled water. Leeches showing no response upon pricking with a needle were considered as dead.

**Method of statistical analysis:** A descriptive statistic was used. The percentage mortality rate data was obtained by dividing the total number of death leeches in each beaker by the total number of leeches exposed. The relationship between mortality rates and concentration of the extract was analyzed using a one-way ANOVA in SPSS Version 20.

## RESULTS AND DISCUSSION

Five phytochemicals, Alkaloids, Flavonoids, Tannins, Terpenes and Saponins were recovered from the four plants. From the extraction, Alkaloids, Tannin and Flavonoids were all present in all four plant extracts. Terpenes were present only in *Costus afer*, *Ficus sur* and *Platostoma africanum* while saponin was present only in *Cassia alata* and *Platostoma africanum* (Fig. 1).

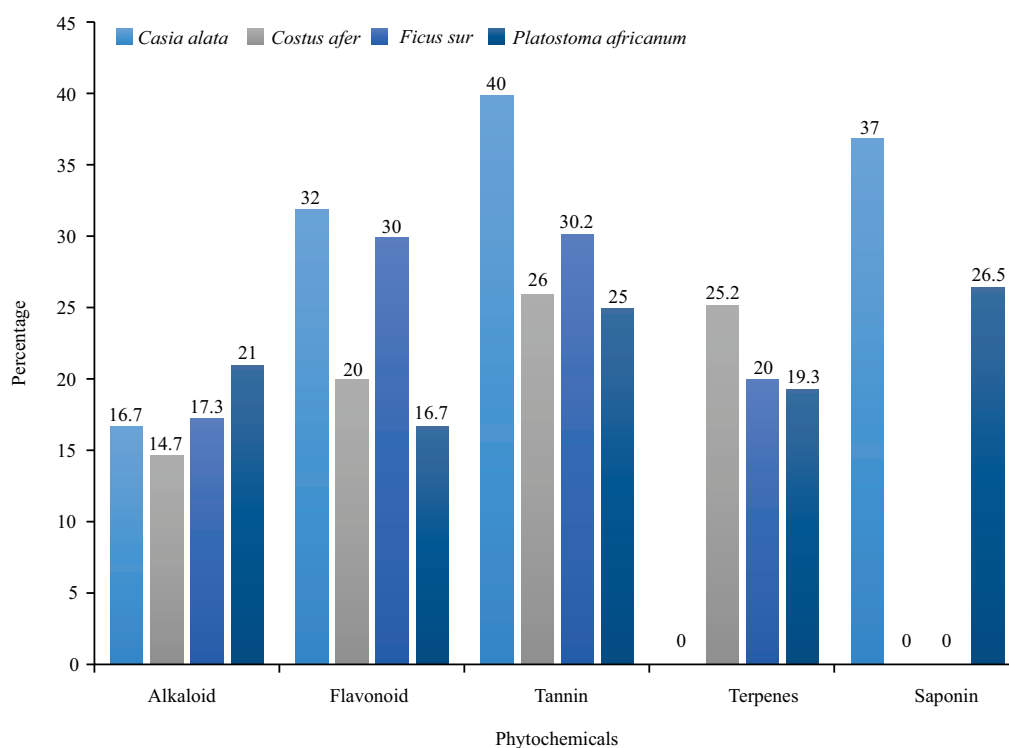


Fig. 1: Express percentage of phytochemicals in the four plant samples

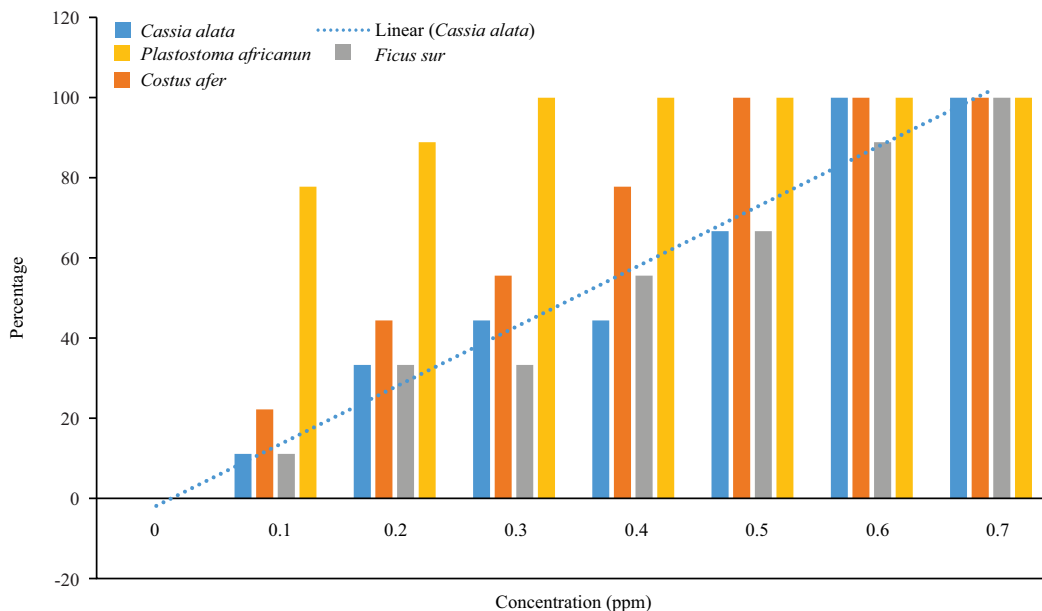


Fig. 2: Trend line in the concentration-dependent mortality rate of the leeches in the four botanicals

Table 1: Mean mortality rates of leeches across extract concentrations

Average mortality rate (%)				
Concentration	<i>Cassia alata</i>	<i>Costus afer</i>	<i>Ficus sur</i>	<i>Platostoma africanum</i>
0.1	11.03 ± 9.11 <sup>a</sup>	22.07 ± 19.11 <sup>a</sup>	11.03 ± 9.11 <sup>a</sup>	47.80 ± 19.22 <sup>a</sup>
0.2	33.10 ± 0.00 <sup>b</sup>	44.39 ± 19.39 <sup>b</sup>	33.33 ± 57.73 <sup>b</sup>	59.27 ± 12.87 <sup>b</sup>
0.3	44.43 ± 19.28 <sup>bc</sup>	44.43 ± 19.28 <sup>b</sup>	55.57 ± 19.28 <sup>c</sup>	100 ± 0.00 <sup>c</sup>
0.4	44.43 ± 19.28 <sup>bc</sup>	77.80 ± 19.22 <sup>c</sup>	55.57 ± 19.28 <sup>c</sup>	100 ± 0.00 <sup>c</sup>
0.5	66.70 ± 0.00 <sup>d</sup>	100 ± 0.00 <sup>d</sup>	66.67 ± 33.35 <sup>d</sup>	100 ± 0.00 <sup>c</sup>
0.6	100 ± 0.00 <sup>e</sup>	100 ± 0.00 <sup>d</sup>	100 ± 0.00 <sup>e</sup>	100 ± 0.00 <sup>c</sup>
0.7	100 ± 0.00 <sup>e</sup>	100 ± 0.00 <sup>d</sup>	100 ± 0.00 <sup>e</sup>	100 ± 0.00 <sup>c</sup>

**Effect of the plant extracts on leeches mortality:** Two hundred and eighty-eight leeches were exposed to the four plants extract at varying concentrations, the mortality rate increases with an increase in the concentration of the plant extracts. The range of the mean and standard deviation mortality rate when compared across the plant extracts and concentration were, *Cassia alata* (11.03 ± 9.11-100 ± 0.00), *Costus afer* (22.07 ± 19.11-100 ± 0.00), *Ficus sur* (100 ± 0.00-11.03 ± 9.11) and *Platostoma africanum* (47.80 ± 19.22-100 ± 0.00), respectively. Differences in the death rate across plant extracts were significant (p < 0.05) (Table 1). The concentration-dependent mortality rate of the leeches in the four botanicals, *Cassia alata*, *Costus afer* and *Ficus sur* and *Platostoma africanum* was trendy (Fig. 2). About 100% mortality was recorded with *Platostoma africanum* at the concentration of 0.3 ppm.

The presence of the four phytochemicals, Alkaloids, Flavonoids, Tannins, Terpenes and Saponins in the four botanicals, *Cassia alata*, *Costus afer*, *Ficus sur* and *Platostoma africanum* has been reported elsewhere<sup>15</sup>. In this present

study, Alkaloids, Tannin and Flavonoids were present in all the four plant extracts, Terpenes was present in *Costus afer*, *Ficus sur* and *Platostoma africanum* while saponin was exclusive in *Cassia alata* and *Platostoma africanum*.

The varying amount and intensity of the phytochemicals in different plants may highlight the differences in the extraction methods<sup>16</sup>. The methanolic extract of *Costus afer* and *Ficus sur* in this study reveals the presence of terpenes in addition to Alkaloids, Tannin and Flavonoids. While this observation is consistent with the report of Ukpabi *et al.*<sup>17</sup> it contrasts the observation. made by Ezejiofor *et al.*<sup>18</sup>, who reported that the aqueous extract of *Costus afer* reveals the presence of saponins, phenols and glycosides in addition to alkaloids, flavonoids, tannins. Anyasor *et al.*<sup>19</sup> and Ukpabi *et al.*<sup>17</sup> all affirmed that aqueous extract of costus after produces a significantly higher dose of flavonoids and saponins than ethanol extract. The exclusiveness of saponin in *Cassia alata* and *Platostoma africanum* is worth researching further.

### Effects of the lethal concentrations of the phytochemicals on the leech mortality:

Phytochemicals extracts of different plants have proven promising patents for the management of various diseases, by their wide range of clinical relevance<sup>20,17</sup>. These potent properties in the plant are the demonstration of the inherent metabolites present in them. The results of the bioassay of the four plant extracts against the leech (*Hirudo medicinalis*) indicates their significant hirudina vital activity. The biological activity of these plant extracts causing the high mortality rate (61.5%) of the leech in this present study may be due to the presence of a wide range of various metabolites such as terpenoids, flavonoids and alkaloids and saponins acting either singly or in combination<sup>21</sup>.

The mortality rate of the leeches varies among the plant samples. The higher mortality rate of leech in the extract from *Platostoma africanum* could be attributed to the combined effect of alkaloids and saponins. According to Kamarul *et al.*<sup>22</sup> alkaloids if in direct contact, swallowed or inhaled is capable of causing a toxic effect on the liver cell and inducing deformities in human muscle. Higher doses of alkaloids may also initiate tissue responses which may trigger a direct effect on human cells. The insecticidal and antiparasitic properties of saponins have been reported<sup>23</sup>. The mortality rate in *Cassia alata*, *Costus afer* and *Ficus sur* was not significant. This observation may be unconnected with similar constituents of metabolites<sup>24</sup>. The Synergistic Potentials of *Platostoma africanum* has been reported by Chinenye *et al.*<sup>5</sup>. The mortality rates varied with the concentration of the plant extract. In *Cassia alata*, *Costus afer* and *Ficus sur*, the mortality rate increases with an increase in the extract concentration. This trend has been reported elsewhere<sup>26</sup>.

### CONCLUSION

The significant mortality activity demonstrated by methanolic extracts of *Cassia alata*, *Costus afer* and *Ficus sur* and *Platostoma africanum* on leeches is a suggestion that the four plants may have strong killing effects against parasites and other related organisms, hence giving a promising hope as hirudina vital agents.

### SIGNIFICANCE STATEMENT

This study discovers five phytochemicals, Alkaloids, Flavonoids, Tannins, Terpenes and Saponins present in four plant extracts, *Costus afer*, *Ficus sur*, *Cassia alata* and *Platostoma africanum*. A bioassay showed that the plant

extracts causes the mortality of freshwater leech, an ectoparasites of fish and amphibians at varying concentrations. This study has uncovered the potentials of *Costus afer*, *Ficus sur*, *Cassia alata* and *Platostoma africanum* extracts in the control of ectoparasites, thus a new approach to parasite control can be arrived at if the potentials of these plant extracts are possibly exploited.

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