

Insecticidal Potentials and Identification of Bioactive Compounds from Extracts of *Bacopa Floribunda* and *Ludwigia Decurrens* Plants

Adewole E. (Corresponding Author)

Department of Chemical Sciences, Industrial Chemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria
Email: adewolen50@yahoo.com

Ogola-Emma E.

Department of Chemistry, Bayelsa Medical University, Bayelsa state, Nigeria

David P.

Department of Chemical Sciences, Industrial Chemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

Peters O.

Department of Chemical Sciences, Industrial Chemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

Oludoro O.

Department of Chemical Sciences, Industrial Chemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

Adewumi F.

Department of Chemical Sciences, Industrial Chemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

Agboola K.

Department of Chemical Sciences, Biochemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

Adejori A.

Department of Chemical Sciences, Biochemistry Programme, Afe Babalola University Ado-Ekiti, Nigeria

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Abstract

The negative impact of insecticides used to combat insect-borne plant ailments cannot be emphasized, as they have had a major influence on plant yields throughout time, and the side effect of insecticide residue on consumers cannot be overstated either. *Callosobrochus maculatus* is an insect that attacks cowpeas and has the potential to significantly reduce crop productivity. This research developed from investigations into the utilization of biological control technologies that have little or no harmful influence on humans or the environment. The leaves of *Bacopa Floribunda* (BFL) and *Ludwigia Decurrens* (LDL) were air dried, powdered, and extracted with n-hexane. The insecticidal activities were carried out according to protocol, and the LC₅₀ was determined using online software from AAT Bioquest, Inc. The presence of bioactive compounds was detected using GC-MS. The *Callosobrochus maculatus* mortality rate was found to be a function of exposure time and concentration of the extracts, with the maximum mortality rate (66.67±8.82 percent) occurring after 72 hours at 0.5 mg/ml for BFL extract. The highest mortality rate (70.00 ±5.77 percent) was obtained for the LDL extract at 72 hours at 0.5 mg/ml of the extract concentration, which was better than the other extract (BFL) at 0.5 mg/ml. The lethal concentration LC₅₀ for BFL varies from 0.251 (24 hours), 0.276 (48 hours), and 0.223 (72 hours), while the LC₅₀ for LDL extract is 0.228 (24 hours), 0.039 (48 hours), and 0.663 (72 hours) (72 hrs). The presence of chemicals found could be linked to the extracts' insecticidal properties. The excellent potential of the extracts as insect biocontrol agents against *Callosobrochus maculatus* can be recommended for future research as insect biocontrol agents.

Keywords: *Callosobrochus maculatus*; *Bacopa floribunda*; *Ludwigia decurrens*; Concentration; Insecticidal; LC₅₀; Mortality; GC-MS.

1. Introduction

Insects and pests have caused unquantifiable economic losses, which has prompted the invention and use of synthetic pesticides for insect control. Farmers' aversion to using these pesticides has resulted in a number of severe consequences, including personal and environmental injuries, prompting them to control the chemicals as reported by Pavela [1], Pavela [2]. Scientists have been overwhelmed with demands to investigate into natural methods of mitigating the detrimental consequences of chemical use, such as biological controls, to minimize the harmful effects of chemical treatments on plants for the express aim of managing pests and insects. Many plant parts have been investigated for their insecticidal activities, and the search for more plants with good insecticidal potential is still on.

Plant preparations, which include powder, extracts, and oils, have been checked for their activities such as fumigants, repellants, and a host of others, as reported by Isman [3], Weaver and Subramanyam [4], Koul [5], Mordue [6], Ertürk [7], Ertürk, *et al.* [8], Negahban and Moharrampour [9], Jbilou, *et al.* [10], Roy, *et al.* [11]. The cowpea weevil, often known as the cowpea seed beetle, is a beetle species called *Callosobruchus maculatus* [12]. Not a genuine weevil, but a leaf beetle belonging to the Chrysomelidae family [12]. This ubiquitous pest of stored legumes has a global distribution and can be found on every continent except Antarctica [13]. The beetle is said to have originated in West Africa and spread throughout the world as a result of the trade in legumes and other crops [12]. Scholars and agricultural institutes have remarked on the unfavourable impact of *C. maculatus* on a number of occasions. *C. maculatus* ate 50-90 percent of cowpea stored in tropical Africa every year, according to the International Institute of Tropical Agriculture IITA (International Institute of Tropical Agriculture) [14]. Farm storage for six months was commonly associated with 70% seed infestation and around 30% weight loss, rendering the food unfit for human consumption [15]. Effect of Biological control of pests and insects have been evaluated by scholars [7, 16-18]. *Ludwigia decurrens* is a dicotyledonous plant that belongs to the Onagraceae family. It is endemic to the Central Eastern United States, but it has spread rapidly throughout the world, naturalizing in aquatic and riparian environments (including rice paddy fields), and is now classified an invasive noxious weed [19]. *L. decurrens* is a fierce competitor for rice and has a significant impact on rice yield [19] and reports has it that it has been in use in Nigeria for centuries to cure a variety of skin, gastrointestinal, wound, and bone joint diseases [19]. Scholars have reported that *L. decurrens* reduces the development of rice plant tillers, panicles, leaves, and spikelets, according to Pessu and Umeozor [19]. As a result, there is a considerable likelihood of an economic impact due to poorer crop yields and quality [20]. The Scrophuliaceae family includes *Bacopa floribunda*. It is a common herb in Ondo State's Ikale/Ilaje villages, Nigeria. It's utilized as a decoction for memory retention and enhancement in both children and adults [21]. In folklore, the leaf of *B. floribunda* is used to treat cognitive impairment. It has been used as a brain tonic in traditional and Ayurvedic medicine for improving memory, anti-aging, and preventing a variety of psychological illnesses [22]. The objective of this study is to examine the insecticidal potentials of the selected *Bacopa Floribunda* and *Ludwigia Decurrens* plants and make appropriate recommendation as biocontrol agent which is highly imperative for food security.

2. Method

2.1. Plant Source

The *Bacopa floribunda* and *Ludwigia decurrens* plants were collected at Ifedapo community, off Afao road, Ado Ekiti, Nigeria in May, 18th 2021. Ado Ekiti, Nigeria is in the Cities location category and is located at 7° 37' 15.9996" N and 5° 13' 17.0004" E in the Nigeria country.

2.2. Crude Extract Preparation

The leaves of the samples were removed, dried at room temperature and blended. Two hundred grams of the powdered samples each were soaked in 2000 ml of hexane for five days, filtered and the extracts were concentrated using water bath. The extracts were labelled for easy identification.

2.3. Insecticidal Activities Procedure

Forty (40) clean cowpea seeds free of insect eggs and properly sterilized at 4°C in a freezer were treated with varying concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg of extracts /ml of hexane of *Bacopa floribunda* leaves (BFL) and *Ludwigia decurrens* leaves (LDL) differently. The solvent used for reconstitution was allowed to dry at laboratory condition. Five pairs of *Callosobruchus maculatus* freshly cultured in the laboratory were introduced into the cowpea seeds that have been previously treated with the extract in a petri-dish and incubated at ambient temperature under laboratory condition. Mortality rates were recorded at 24, 48 and 72 hours after incubation. Oviposition was recorded after 72 hours of incubation with the aid of magnifying eyes level. The % mortality could be transformed by using Arc-sin. Transformed. The % oviposition could be transformed by square-root transformation formula. $\text{Arc-sin} = \sqrt{(x/100)\%}$; where, x= average percentage mortality ; $\text{SR} = \sqrt{x}$; where x= average % oviposition [23].

2.4. Statistical Analysis

The results were done in triplicate and the results were expressed as mean and standard deviation (S.D).

LC₅₀: The lethal concentration (LC₅₀) was calculated using the online software AAT Bioquest, Inc. (2022, January 06). Quest Graph™ LC₅₀ Calculator (v.1)." Retrieved from <https://www.aatbio.com/tools/lc50-calculator-v1>.

2.5. Identification of the Compounds Using GC-MS

The compounds present were revealed by the use of gas chromatography -mass spectrophotometer, the data were obtained by collecting the mass spectra within the scan range 50-550 m/z. The mass spectra were matched with that of the standards available at NIST library.

3. Results and Discussion

3.1. Results

Table-1. Insecticidal activities of *Bacopa floribunda* leaves extract (BFL)

Concentration (mg/ml)	Mortality rate at 24 hours (%)	Mortality rate at 48 hours (%)	Mortality rate at 72 hours (%)	Oviposition	Oviposition without treatment
0.1	6.67 ± 3.33	13.33 ± 3.33	26.67 ± 3.33	73.00 ± 6.09	157
0.2	13.33 ± 3.33	26.67 ± 3.33	43.33 ± 3.33	69.67 ± 5.05	166
0.3	23.33 ± 3.00	43.33 ± 3.33	56.67 ± 3.33	44.00 ± 2.89	133
0.4	30.00 ± 5.77	53.33 ± 6.67	66.67 ± 6.67	38.33 ± 3.29	
0.5	30.00 ± 5.77	60.00 ± 5.77	66.67 ± 8.82	35.00 ± 2.31	
LC ₅₀	0.251	0.276	0.223	0.259	0.023

Table 2. Insecticidal activities of *Ludwigia decurrens* leaf (LDL) extract

Concentration (mg/ml)	Mortality at 24 hours (%)	Mortality rate at 48 hours (%)	Mortality rate at 72 hours (%)	Oviposition	Oviposition without treatment
0.1	10.00 ± 5.77	13.33 ± 6.67	23.33 ± 6.67	73.00 ± 6.09	157
0.2	16.67 ± 3.33	33.33 ± 6.67	46.67 ± 8.82	69.67 ± 5.05	166
0.3	26.67 ± 3.33	33.33 ± 3.33	50.00 ± 10.00	44.00 ± 2.89	133
0.4	30.00 ± 5.77	40.00 ± 0.00	66.67 ± 6.67	38.33 ± 3.29	
0.5	30.00 ± 5.77	43.33 ± 3.33	70.00 ± 5.77	35.00 ± 2.31	
LC ₅₀	0.228	0.039	0.663	0.259	0.023

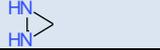
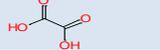
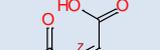
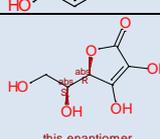
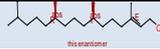
Table-3. Some of the identified compounds in the chromatogram of *Bacopa floribunda* leaves extract,

Peak	Retention Time (minutes)	Compound Name
9	13.222	Hexadecanoic acid, methyl ester
10	13.973	1-(+)-Ascorbic acid 2,6-dihexadecanoate
13	17.543	Phytol
15	18.547	Stearic acid
18	20.293	2(1H)-Pyridinone
25	23.200	Trimethyl diaziridine
27	23.873	Cyclooctasiloxane, octadecamethyl-
33	26.571	Squalene
34	27.148	Alpha tocopherolquinone
37	28.791	Cyclononasiloxane, octadecamethyl-

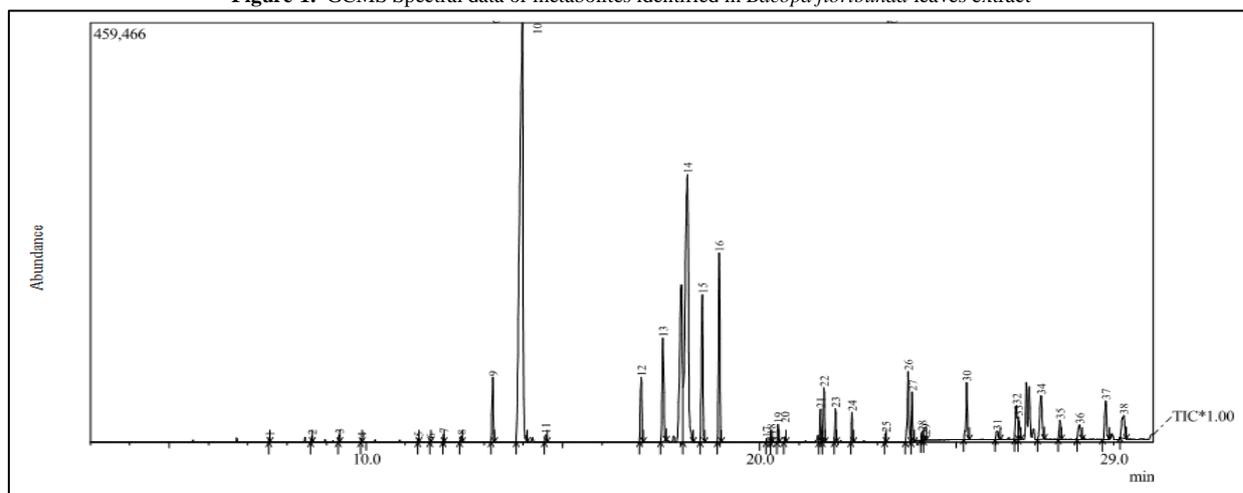
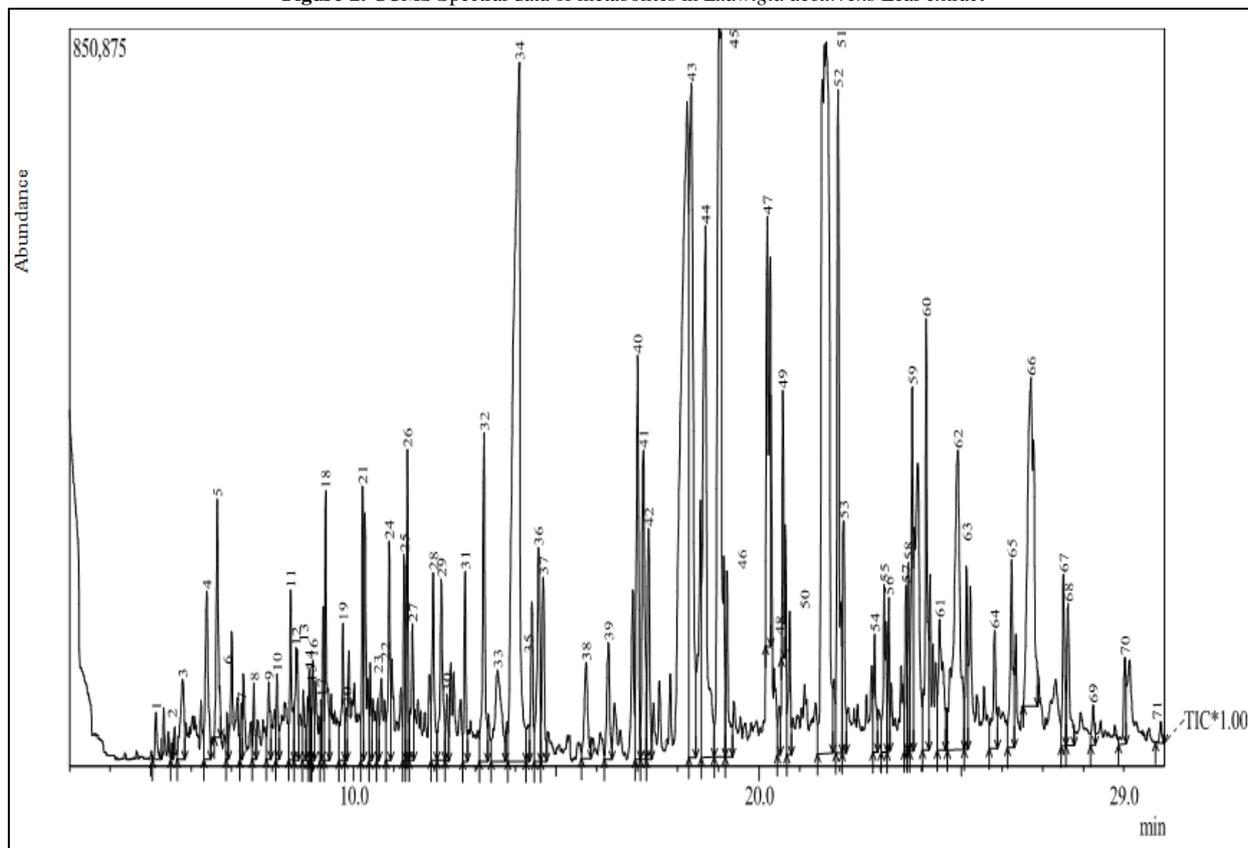
Table-4. Some of the identified bioactive compounds in the *Ludwigia decurrens* leaf extract

Peak	Retention Time (minutes)	Compound Name
2	5.520	Fumaric acid
8	7.545	Oxalic acid
9	7.913	Pentobarbital
15	8.962	Eicosanoic acid, Arachidic acid
21	10.226	Eicosane
28	11.968	Dioxirane
32	13.220	Palmitic acid, ethyl esters
34	14.088	1-(+)-Ascorbic acid 2,6-dihexadecanoate
35	14.317	Myristic acid
45	19.018	Palmitic acid, butyl esters

Table 5. Chemistry of some of the identified compounds in *Ludwigia decurrens* (LDL) and *Bacopa floribunda* Leaves extracts (BFL)

Compound Names	Chemical structure	H-Acceptor	H-Donors	Non-H-atom	Non C/H-atom	SP ³ -atom	Rotatable bond	Electronegativity
Stearic acid		2	1	20	2	18	16	2
diaziridine		2	2	3	2	3	0	2
Oxalic acid		4	2	6	4	2	1	4
Fumaric acid		4	2	8	4	2	2	4
Ascorbic acid		6	4	12	6	8	2	6
Phytol		1	1	21	1	19	13	1

Source: [24].

Figure-1. GCMS Spectral data of metabolites identified in *Bacopa floribunda* leaves extract**Figure-2.** GCMS Spectral data of metabolites in *Ludwigia decurrens* Leaf extract

4. Discussion

The effectiveness of any biocontrol agent depends on the dosage concentrations of solution adopted for the control of insects and pests and the exposure rate (hours). According to the results of table 1, the mortality rate of BFL at 0.1 mg/ml was at a range of $6.67 \pm 3.33\%$, $13.33 \pm 3.33\%$ at 0.2 mg/ml, and this increased down the column up to $30.00 \pm 5.77\%$ at 0.5 mg/ml, all after 24 hours of incubation. At 48 hours, the mortality rate increased from $13.33 \pm 3.3\%$ at 0.1 mg/ml to $60.00 \pm 5.77\%$ at 0.5 mg/ml. This trend was witnessed after 72 hours, when the mortality rate increased from $26.67 \pm 3.33\%$ at 0.1 mg/ml to $66.67 \pm 8.82\%$ at a concentration of 0.5 mg/ml. The lethal concentration dose, LC_{50} , was highest at 48 hours (0.276). The ovi-position decreased as concentration increased from 0.1 mg/ml to 0.5 mg/ml, and this was better than the ovi-position without treatment. Furthermore, from the results of table 2, the mortality rate also increased as the concentration increased from 0.1 mg/ml and the duration of exposure (hours). The highest mortality rate ($70.00 \pm 5.77\%$) was recorded at a concentration of 0.5 mg/ml after 72 hours. The LC_{50} (0.663) was highest at 72 hours and the ovi-position also decreased from $73.00 \pm 6.09\%$ to $35.00 \pm 2.31\%$. In another development, the insecticidal activities of plant extracts may not be unconnected to the presence of bioactive components as revealed by the use of GCMS. From figure 1, thirty-eight bioactive compounds were identified in the chromatogram of *Bacopa floribunda* leave extracts, and when compared to figure 2, seventy-one compounds were revealed in the chromatogram of *Ludwigia decurrens* leave extract. Some of these compounds were identified to possess various insecticidal potentials. According to the findings of these studies, from result of table 3, compounds such as diaziridine derivatives, phytol, and squalene have been reported to have insecticidal properties [25-27]. Also from table 4, the insecticidal potential of the LDL extracts may be due to the presence of some of the identified compounds such as dioxirane, fumaric acid, oxalic acid, and myristic acid, which have all been reported to possess various insecticidal activities [28-31]. From the results of table 5, the chemistry of some of the identified compounds was revealed, such as the number of rotatable bonds, electronegativity, H-acceptor, and chemical structure using Osiris Online server [24].

5. Conclusion

The insecticidal activity of the plants, their LC_{50} , their bioactive components, and the general chemistry of the discovered compounds were all revealed in this study. The two plants, it can be inferred, have a lot of potential as insecticides.

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