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Larvicidal Activity, Phytochemical and GC-MS Analysis of Crude Extract derived from *Ceriop decandra* (Griff) against *Aedes aegypti* (Linnaeus)

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Abstract

Mosquito borne diseases are on increase, couple with increasing effects of chemical control agents which is leading to environmental pollution. This necessitated the use of natural and eco-friendly sources for mosquito control. Record from local inhabitants have shown that certain shrubs and herbs can effectively control mosquito infestations. This present research screened the leaf, bark and root extract of Ceriops decandra (Griff) a mangrove specie from the state of Terengganu in Malaysia against third instar larvae of Aedes aegypti. Screening was carried out in accordance with World Health Organization (WHO) protocol of GC-MS analysis having 39 displayed metabolites from the leaf with 2-pyrrolidinone, 1-methyl as the major constituent, 25 metabolites from the bark with 4-O -methyl mannose as its major constituent. The root has 23 metabolites with 1-propene, 3-[(4nitrobutyl) thio as the major constituent, the probit analysis of the three plant parts showed that the leaf has the highest value with mortality of 83% to larvae with lethal concentration value of LC50 34.27±0..90 ppm, the root has 76% mortality and LC50 of 45.13±1.00 ppm and bark has the least value with 60% mortality rate of larvae, LC50 78.20±1.02 ppm. The result showed that the plant could play a major role as a sustainable and alternative control in place of chemical insecticides.

Keywords: Mangroves, Larvicidal, Phytochemical, Medicinal, Lethal Concentration *Ceriops decandra, Aedes aegypti*.

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INTRODUCTION

Since the beginning of time, mosquitoes have posed a threat to human health. Mosquitoes are vectors of common illness such as malaria, Yellow fever, Zika and Dengue fever which are some of the most prevalent arboviral diseases worldwide. Dengue fever is transmitted by *Aedes aegypti*, a mosquito species that can also vector chikungunya, Zika fever, and yellow fever viruses (Hemisphere, 2020). Aedes mosquitoes have been described as insects having a lyre-shaped marking on its top thorax; and black and white markings on its legs (Goddard and Goddard, 2018; Roy and Das, 2024). One of the most effective strategies of controlling Aedes mosquito is the prevention of their breeding, thereby destroying the immature stages, often regarded as container breeders, Aedes mosquitoes prefer artificial breeding sites, although this preference can be reversed, as they have evolved so many reproductive strategies especially in most challenging environment (Jorge *et al*., 2019).

A number of synthetic larvicides have been effective against Aedes mosquito larvae, such as pyriproxyfen, temephos (Wang *et al*., 2013; Lamaningao *et al*., 2020). However, the challenge of harmful non-biodegradable leftovers polluting the ecosystem and negatively affecting wildlife, the pervasive emergence of vector resistance to synthetic insecticides, and having an adverse effect on species other than the target has prompted fresh interest in using natural products for pest control (Jantan *et al*., 2003). As well as available World Health Organization data on the incidence of mosquito related outbreaks such as Malaria, from Nigeria (31% mortality) and Africa in general (94% mortality) necessitated further probe into its solution in an environmentally friendly approach (Venkatesan, 2024). The benefits of plant-based insecticides over modern synthetic or chemical pesticides come from their compositions of natural mixtures of various chemical components that could have a combined impact on mosquitos' physiology and behaviours (Ghosh *et al*., 2012). Biologically active substances are well-preserved in the maritime environment. Mangrove being among the most abundant sources of floral and its variety (Gnanadesigan *et al*., 2017) has a unique biochemical makeup and contain numerous innovative organic molecules or compounds. Antiviral, antibacterial, and antifungal chemicals with biological activity can be found in mangroves and their companions (Shelar *et al*., 2012).

Although, plants create and store a huge number of primary and secondary organic compounds, it is the secondary organic compounds that are more important for natural products because they are the source of physiologically active molecules. Depending on the environment, plants' chemical composition, concentration, and localisation change, types and sources (Morales-Covarrubias *et al*., 2019). According to several studies, flavonoids possess the following capacities; anti-hypertensive actions, anti-cancer, anti- oxidant and anti-inflammatory among others. Tannins act as insecticides because they inactivate proteins. Astringent qualities are another quality they have. The polio virus, herpes simplex, and other intestinal viruses have all been demonstrated to be inactivated by tannins. Saponins reduce the risk of cardiovascular disease and cholesterol levels and act as natural antibiotics (Korfii *et al*., 2022). In addition to being new sources of antiviral, antibacterial, antifungal, and insecticide for pharmaceutical application, many mangrove species include bioactive metabolites and chemicals that govern microbial growth. These compounds can be used as an alternative therapy in aquaculture because their extracts have powerful pathogen-inhibitory action (Bobbarala *et al*., 2009). Alkaloids, flavonoids, tannins, saponins, carotenoids, etc. are a few of the phytochemicals that have been used extensively in medicine. For instance, many alkaloids are very poisonous and may have harmful effects on the nervous system, muscles, or membranes. As narcotics, anesthetics, antimalarials, stimulants, and insecticides, alkaloids (morphine, codeine, and cocaine) are employed often. The anti-inflammatory, anti-allergic,

and anti-cancer properties of flavonoids are also crucial (Korfii *et al*., 2022). Different portions of these mangrove plants have been used in various therapeutic conditions since the dawn of human civilisation, either voluntarily or unconsciously. The majority of mangrove plants possesses bioactive components, like antioxidants, which are useful to mention but little (Habib *et al*., 2018). Mangrove plant extracts have been documented for several decades to treat a variety of medical conditions. Due to their many uses, chemicals derived from plants have recently attracted a lot of attention. Additionally, mangroves produce a variety of non-timber goods like tannin, fish poison, medicine, food, and fodder (Harkulkar, 2015). Depending on the environment, plants' chemical composition, concentration, and localization changes (Morales-Covarrubias *et al*., 2019). The variability is due to the requirements for adaptation in unique environment (Numbere, 2018; Srikanth *et al*., 2016).

Mangrove plants possess both primary and secondary organic compounds, however it is the secondary organic compounds that are more important for natural products because they are the source of physiologically active molecules. They are rich in Tannins, a substance that act as insecticides because they inactivate proteins. Different portions of these mangrove plants have been used in various therapeutic conditions since the dawn of human civilisation, either voluntarily or unconsciously. Majority of mangrove plants possesses bioactive components, like antioxidants, which are useful to mention but little (Habib *et al*., 2018; Nugroho *et al*., 2020). Mangrove plant extracts have been utilized for many years to treat a variety of medical conditions. The use was associated with the availability of essential nutrients, including proteins, carbohydrates, and amino acids, which are needed for the upkeep of biological processes as well as bioactive substances (Kalasuba *et al*., 2023).

Ceriops is a genus of mangrove of the family Rhizophoraceae with five (5) known species and they have very few genetic diversity and fewer haplotypes at the population level (Huang *et al*., 2008). Several studies have demonstrated that numerous different phyto-constituents, such as diterpenoids (ceriopsin A-G), triterpenoids (lupeol, a-amyrin, oleanolic acid, ursolic acid), and phenolics (catechin, procyanidins), are abundant in many plant tissues (Kumar *et al*., 2013). Species include but not limited to *Ceriops decandra*, *C. tagal*, and *C. australis*. *Ceriops decandra* has been reportedly useful in the management of digestive disorders and fungal infection (Mahmud *et al*., 2019). Also, its latent quality as insecticide have been reported (Kumar *et al*., 2013; Zhao *et al*., 2021). Until now, very few studies have reported on the insecticidal potentials of this plant. This study presents a checklist of the constituents of methanol extracts of *Ceriops decandra* leaf, bark and, root as well as compare the insecticidal potentials of different parts of the plants extract against third instar larvae of *Aedes aegypti*.

MATERIALS AND METHODS

Plant Materials Collection, Preparation, and Extraction: Fresh whole *Ceriops decandra* (Grif) plant were collected from Setiu wetland, located on longitude 1020 82.74.0671 and latitude 050.45.47 400 (Kuala Terengganu). The voucher specimen was identified and deposited at the Herbarium of the Institute Marine Biotechnology Universiti Malaysia Terengganu. The harvested parts (Leaf, Bark, Root) of plant were washed separately under tap water and later washed with distilled water after which they were separately air dried until its properly dried (for two weeks). Three hundred grams of finely grounded powder of each plant parts were soaked in 500ml of methanol at room temperature and concentrated to dryness using Buchi rotary evaporator to get the dry extract.

Gas Chromatography-Mass Spectroscopy Analysis: A Gas Chromatograph coupled to a Mass Spectrometer (GC-MS) outfitted with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column (30 0.25m ID 0.25 m df) was used to analyze the methanolic extract of the various plants. An electron ionization system was used for GC-MS detection, and the ionization energy was set at 70 eV, with the system operating in electron impact mode. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1). The injector was kept at 250 ^{o}C, while the ion source was kept at 200 ^{o}C, and the oven temperature was raised from 110 ^{o}C (isothermal for 2 min) to 200 ^{o}C (isothermal for 5 min), and finally to 280 ^{o}C (isothermal for 9 min). With a scan interval of 0.5 s and fragments ranging in size from 45 to 450 Da, mass spectra were collected at 70 eV. The GC/MS ran for a total of 36 minutes, with a solvent delay of 0 to 3 min.

Larvicidal Bioassay- Collection and Rearing of Mosquito larvae: Collection and rearing of Mosquito larvae for bioassay test was conducted in the Entomology Laboratory of the Department of Zoology and Environmental Biology, Lagos State University, Ojo. The larvae of *A. aegypti* were collected from abandoned drums, used tyres and gutters in different parts of Lagos metropolis. Rearing of the Larvae was carried out under atmospheric temperature 28 ± 10^{o}C and 68 ± 2 % relative humidity. The colony of the mosquitoes was maintained in accordance with the procedure of Anyaele and Amusan (2010). Weight /volume stock solution was prepared using World Health Organisation [WHO] (2010) guideline/ test procedure for larvicides. Leaf extracts were diluted with Methanol at 0.001g of powder to 0.001ml per 100ml of Methanol. The same admixture rate was used for the bark and root extracts. After which varying concentrations were prepared for the three parts: 100ppm, 80ppm, 20ppm and 5ppm to test the larvicidal activity. Thirty larvae (3rd instar) of *A. aegypti* were transferred by means of a loop into a separate plastic bowl containing 150 mL of distilled water, after which 100ml of the prepared concentrations was then added. The samples were prepared in triplicate according to the concentrations 5ppm, 20 ppm, 80ppm, 100 ppm and a control bowl. Larvicidal activity, was examined using extracts from *C. decandra* leaves, bark and root.

Statistical Analysis: The lethal concentrations (LC) was calculated using probit program version 2000 to calculate LC_{50} LC_{90} and LC_{99} concentrations and the data were analyzed using Statistix version 10. Means were determined and compared using oneway analysis of variance (ANOVA) and separated according to Least Significant Difference (LSD) at 5% level of significance. All data collected were in triplicate.

RESULTS AND DISCUSSION

GC-MS Phytochemical Analysis of *Ceriops decandra*: The GC-MS analysis of the leaf, bark, and root extract of *Ceriops decandra* from setiu wetland Terengganu Malaysia was found to contain various classes of phytoconstituents. The methanol extract of the leaf is found to contain thirty-nine various constituents and the major constituent of the leaf is 2-pyrrolidinone, 1- methyl at retention time of 4.066 mins with percentage area of 49.66% (Table 1). The major constituent of the Methanol bark extract is 4-O- Methyl mannose at retention time of 11.899 mins with an area of 82.30%, it contains twenty-five components in all (Table 2). These results further corroborate the effects of location and genetic diversity on proportion and constituent of mangrove species such as *Ceriops* species (Yan *et al.*, 2016; Sadeer *et al.*, 2023; Ramesh *et al.*, 2024).

The root contains twenty-three constituents, the major root constituents being 1-Propene, 3-[(4-nitrobutyl) thio]- at retention time of 11.865 with an area of 60.86% which is followed by

catechol at retention time of 6.463mins and area of 12.73% and Benzoic acid methyl ester retention time of 4.787mins and an area of 6.16% (Table 3). Catechol a substance that has been reported for prolonging pesticide retention and flush resistance on foliage (Vundru *et al.*, 2013).

Table 1: Chemical Constituent of Methanol Extracts of *Ceriops decandra* Leaf

S/n	Rt	Identified Compound	Area%
1	3.431	Benzene, 1,2,3-trimethyl-	1.70
2	3.476	Benzene, 1-ethyl-3-methyl-	5.25
3	3.814	2,3-Heptadien-5-yne, 2,4-dimethyl-	0.54
4	4.066	2-Pyrrolidinone, 1-methyl	49.66
5	4.741	Decane	3.76
6	6.017	Oxirane, [(dodecyloxy)methyl]-	0.75
7	6.801	Decane, 3-ethyl-3-methyl-	0.84
8	6.996	Dodecane, 2,6,10-trimethyl-	2.51
9	7.333	trans-2,3-Epoxydecane	0.53
10	7.539	Dodecane, 2,6,10-trimethyl-	1.09
11	8.375	9-methylheptadecane	3.30
12	8.615	Carbonic acid, nonyl prop-1-en-2-yl ester	0.56
13	8.792	Hexane, 1-(hexyloxy)-5-methyl-	0.51
14	8.947	Oxalic acid, allyl nonyl ester	0.62
15	9.044	Dodecane, 2,6,10-trimethyl-	1.33
16	9.359	Heneicosane, 3-methyl-	1.64
17	9.404	Nonane, 3-methyl-5-propyl-	2.03
18	9.702	2,4-Di-tert-butylphenol	2.69
19	9.765	Benzoic acid, 5-azido-2-nitro-, 5- (3,3-dimethyloxiranyl)-3-methyl- pentenyl ester, (E)-.+/-	0.52
20	9.862	Tetradecane, 2,6,10-trimethyl-	1.60
21	10.452	Undecane	1.84
22	10.515	trans-2,3-Epoxyoctane	0.74
23	10.801	Carbonic acid, decyl tridecyl ester	0.59
24	10.955	Carbonic acid, decyl undecyl ester	0.57
25	11.161	Dodecane, 1-fluoro-	0.54
26	11.287	2-Methyltetracosane	0.56
27	11.424	Methoxyacetic acid, 3-tetradecyl ester	0.61
28	11.504	Heptacosane	2.30
29	11.899	Sulfurous acid, butyl heptadecyl ester	1.20
30	12.317	Sulfurous acid, butyl heptadecyl ester	0.68
31	12.672	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin	0.58
32	12.746	Sulfurous acid, 2-propyl tetradecyl ester	0.60
33	12.992	7H-Purin-6-amine, 7-methyl-	0.52
34	13.107	Sulfurous acid, dodecyl 2-propyl ester	0.61
35	13.181	Oxalic acid, cyclobutyl tridecyl ester	0.60
36	13.410	Tetradecanoic acid, 12-methyl-, methyl ester	3.30
37	14.491	beta. -d-Lyxofuranoside, thio-heptyl-	0.72
38	15.012	Dodecanoic acid, 10-methyl-, methyl ester	1.31
39	16,345	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	0.69

Table 2: Chemical Constituent of Methanol Extracts of *C. decandra* Stem Bark

S/N	Rt	Identified Compound	Area%
1	3.413	Oxalic acid, cyclobutyl decyl ester	0.32
2	3.791	13-Tetradecynoic acid, methyl ester	0.10
3	4.140	Phenol, 3-methyl-	0.25
4	4.724	Decane	1.04
5	4.787	Benzoic acid, methyl ester	5.48
6	5.084	Nonanoic acid, 9-oxo-, methyl este	0.28
7	6.366	2-Cyclopropylcarbonyloxypentadecan	0.32
8	6.566	2,4,6-Cycloheptatrien-1-one, 4-methyl	0.10
9	6.715	9-Oxabicyclo[3.3.1] nona-2,6-diene	0.10
10	8.329	Benzene, (azidomethyl)-	0.21
11	8.426	Phenol, 4-(ethoxymethyl)-	0.14
12	8.992	3-Bromomethyphenol	0.20
13	9.215	2-(2-Hydroxyphenoxy)-1-phenylethanol	0.18
14	10.068	Propionic acid, 3-(allylthio)-, sec-butyl ester	0.10
15	10.394	Card-20(22)-enolide,3-[(2,6-dideoxy-4-O-.beta.-D-glucopyranosyl-3-O methyl-.beta.-D-ribo-hexopyranosyl)oxy]-5,14-dihydroxy-19-oxo-,(3.beta.,5.beta.)	0.15
16	11.035	3-Methylmannoside	0.08
17	11.333	Silane, ethyltrimethyl-	1.47
18	11.899	4-O-Methylmannose	82.30
19	12.935	Alanyl-.beta.-alanine, TMS derivative	0.45
20	12.992	1-Hydroxy-2,2,5,5-tetramethyl-4-(p-fluorophenyl)-3-imidazoline-3-oxide	0.25
21	13.227	Methoxydiethoxyphosphine	0.30
22	13.410	Pentadecanoic acid, 14-methyl-, methyl ester	3.61
23	14.217	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	0.14
24	14.829	7-Hexadecenoic acid, methyl ester, (Z)-	0. 88
25	15.012	Methyl stearate	1.55

Table 3: Chemical Constituent of Methanol extracts of *C.decandra* Root

S/n	Rt	Identified Compound	Area%
1	3.142	Boronic acid, ethyl-, bis (2-mercaptoethyl ester)	0.56
2	4.180	Isobutyl 3-hydroxy-2-methylenebutanoate	0.32
3	4.724	Heptane, 2,6-dimethyl-	0.93
4	4.787	Benzoic acid, methyl ester	6.16
5	6.463	Catechol	12.73
6	7.550	1,2-Benzenediol, 4-methyl-	2.31
7	7.968	Phenol, 2,6-dimethoxy-	1.55
8	8.294	7-Hexadecene, (Z)-	0.78
9	8.964	3-Butyn-2-one, 4-[3,3-dimethyl-2- 1-methylethyl)oxiranyl]-	0.32
10	9.216	Trichloroacetic acid, 2-methyloct- 5-yn-4-yl ester	0.42
11	9.708	2,4-Di-tert-butylphenol	2.41
12	10.080	Succinic acid, butyl 3-methyl-2-nitrobenzyl ester	0.36
13	10.383	5-Octadecene, (E)-	0.96
14	10.749	3,4,5-Trimethoxyphenol	0.66
15	11.865	1-Propene, 3-[(4-nitrobutyl)thio]-	60.86
16	12.260	1-Docosene	1.08
17	12.998	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.77
18	13.410	Pentadecanoic acid, 14-methyl-, methyl ester	1.26
19	13.787	Dibutyl phthalate	0.68
20	13.953	2-Heptafluorobutyroxypentadecane	0.41
21	15.021	Methyl stearate	0.91
22	15.458	Hexadecanoic acid, 1,1-dimethylethyl ester	1.70
23	15.556	(2-Iodo-phenyl)-carbamic acid isobutyl ester	0.89

Larvicidal Effect of Extracts of *C. decandra* Against 3rd Instar Larvae of *Aedes Aegypti*: *Ceriops decandra* exhibited high larvicidal potentials on Aedes mosquito larvae. The larvicidal effects of both methanol extracts of *Ceriops decandra* (leaf, bark, and root) against the 3rd instar larvae of *A. aegypti* is shown in Table 4 and Figure 1. The LC50 value of the leaf (CL95%) is 34.27±0.90ppm and LC90 (CL95%) is 147.01±1.64 ppm while the LC99 is 502.44±1.97 ppm. The LC50 for the Bark (CL95%) is 78.20±1.02 ppm, LC90 (CL95%) 469. 40±0.62 ppm, while LC99 is 2015.80±1.00 ppm. The Lethal Concentration LC50 (CL95%) of the root extract was 45.13±1.00ppm, LC90 (CL95%) 235.26±0.90ppm, LC99 is 885.52±1.470ppm. Also, the leaf extracts had the highest mortality of 83% compared to others (Figure 1). Thus, the leaf extract was more potent requiring less quantity as compared with the roots, while the bark extracts were the least potent. Chloroform extracts of *Ceriops decandra* leaves tested against 3rd instar *Aedes aegypti* also recorded a LC50 value of 251.2% (Vundru *et al*., 2013) while Ali *et al* (2014) also established the insecticidal potentials of *Ceriops decandra* leaves amongst other plants of the family Rhizophoracaea on 4th instar larvae of *A.aegypti* recording LC50 value of 0.0892 ± 0.0063.

Table 4: Lethal Concentrations of *C. decandra* on Third instar Larvae of at 24 hours exposure

Plant Parts	LC50 (ppm)	LC90 (ppm)	LC99 (ppm)
Root	45.13±1.00^{b}	235.26±0.90^{b}	885.52±1.470^{b}
Bark	78.20±1.02^{a}	469.40±0.62^{a}	2015.80±1.00^{a}
Leaf	34.27±0.90^{c}	147.01±1.64^{c}	502.44±1.97^{c}

Triplicate values are presented as Mean±Standard Deviation; LC= Lethal Concentration; Means with different superscripts on the same column are significantly different at 5% probability level according to LSD.

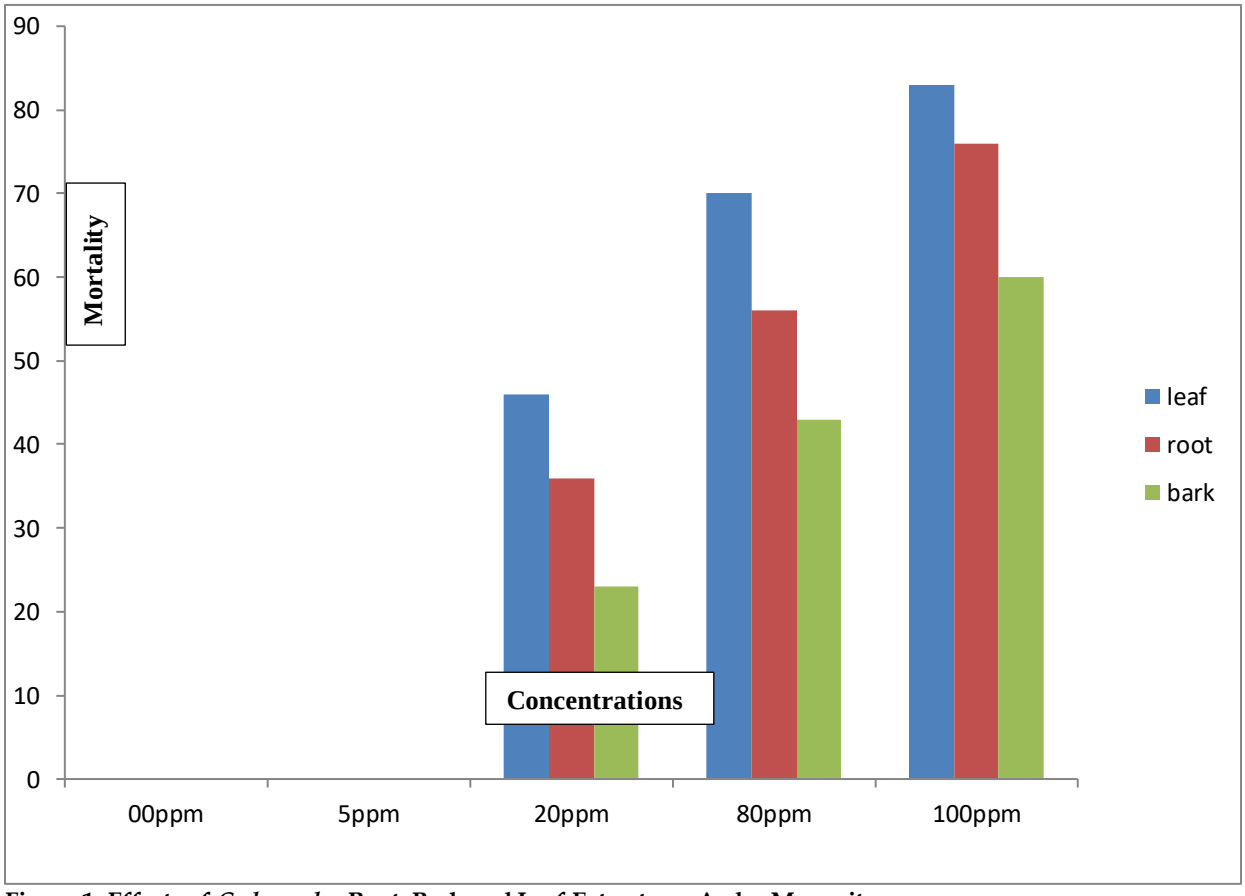

Figure 1: Effects of *C. decandra* Root, Bark and Leaf Extracts on Aedes Mosquitoes

CONCLUSION

The *Ceriops decandra* crude extract has larvicidal effects on the tested mosquito species. Most of the time, synergistic interactions with small components are likely to blame for the larvicidal activities, which cannot typically be assigned to the major components. The leaf had the highest mortality rate, followed by the root and then the bark, all of which showed potential. Therefore, the essential oils from this mangrove species may act as "green" vector control agents and/or complementing agents, as well as provide value-added commodities for harvested food and other items.

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