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Molluscicidal and Synergicidal Activities of the Leaves of Four Niger Delta Mangrove Plants Against Schistosomiasis Vectors

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Abstract

Plant-derived pesticide have found several remedial application in the control of most diseases (especially vector-borne diseases). Synthetic pesticides are unaffordable in most disease endemic areas; besides they may induce varying toxicity to non-targeted species as they bio-magnify along the food-chain. The fecundity of vector/parasites in the Niger Delta is becoming hyper-endemic due to the water-logged nature of the terrain. The molluscicidal activities of crude and methanolic extracts of the leaves of four Niger Delta Mangrove plants (Rhizophora mangle, R. racemosa, Avicennia germinans and Languncularia racemosa), were investigated against some vectors of schistosomiasis (Biomphalaria pfeifferi, Bulinus globosus and Bulinus rholfsi), following standard protocol. Compared to the individual activities demonstrated by the four plants, the highest mortality was recorded in the methanolic-synergicidal formula (1:1:1:1), of the four plants against B. pfeifferi (LC50=74.84ppm), B. globosus (LC50=65.10ppm) and B. rholfsi (LC50=28.00ppm). Also, within the synergicidal results, *B. pfeifferi* was less susceptible to all extracts compared to B. globosus and B. rholfsi. These results indicate that Niger Delta Mangrove plants show great potential as molluscicide for the control of schistosomiasis.

Keywords: Niger Delta, Mangrove plant, Molluscicide, Schistosomiasis

1 Introduction

Schistosomiasis is a vector-borne disease caused by parasites of the genus *Schistosoma* [1], which are hosted and transmitted by minute aquatic snails (10±2mm), belonging to several genera (*Biomphalaria*, *Bulinus*, *Oncomelia* e.t.c.). The intermediate carrier snails of Schistosomiasis are found in fresh water bodies [1, 2]. It has been reported that more that 4% of the world's population are at risk of schistosomiasis [3]. The sail borne dreadful trematode causes infections in human, wild animals and domestic animals [2, 4], in different part of the world including Africa, Caribbean, Asia, Middle East and South America [4, 5]. Schistosomiasis is endemic in 70 - 74 countries affecting approximately 200 to 207 millions of people globally [4, 6, 7], with 120 million and 20 million of these having symptomatic and severe disease conditions respectively [4, 8].

Specifically, *Schistosoma mansoni* is endemic in 54 countries [9], and it is estimated to infect more than 83 million people worldwide [10]. Human schistosomiasis, is a chronic and devastating parasitic disease, which is ranked second after malaria in terms of public health significance [11]. Human pathogenic species of schistosomes includes intestinal schistosomiasis caused by *Schistosoma mansoni* (with intermediate host being *Biomphalaria glabrata*, *Biomphalaria alexandrina*, *Biomphalaria pfeifferi*), while *Schistosoma intercalatum*, *Schistosoma japonicum and Schistosoma mekongi* has *Bulinus forskali*, *Oncomlenia hupensis* and *Tricula aperta* respectively as intermediate host and *Schistosoma haematobium* which urogenital schistosomiasis has *Bulinus truncates* and *Bulinus globosus* intermediate host [4]. Mortality rate due to schistosomiasis was estimated to be 15,000 deaths per year, excluding indirect mortality associated with schistosomiasis infection consequences such as liver disease, portal hypertension, haematemesis, non-functioning, kidney, cervical and squamous cell bladder carcinoma [12]. Different species of *Schistosoma* inhabit in different part of the body. For instance, *S. mansoni* parasites which is a digenetic trematode platyhelminths inhabits in the mesenteric veins of host animals such as humans [7].

Schistosomiasis is widespread in developing countries [2] such as Egypt, Nigeria to mention just a few, intestinal schistosomiasis is common, recurrent and has been described as a long-lasting health problem [13]. Basically, Nigeria is one of the developing countries with the highest number of people infected with Schistosomiasis [14]. There is no vaccine available for the treatment of schistosomiasis at as 2001, and chemotherapy is use to combat the disease

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[11]. Praziquantel is the most widely employed drug, but challenged with the problem of rapid rates of re-infection subsequent to treatment [1, 6, 11, 14]. Other drugs such as Oxamniquine has become problematic to obtain and even metrifonate has recently been withdrawn from the market [11]. This and many more reasons have necessitated the need for a multifaceted approach such as plant-derived phytochemicals in the management and control of this disease [1, 15, 16].

Mangrove plants are halophytic plants with highly specialized morphological and physiological features for adaptations, which cover an entire global area of over 15 million ha [3, 17]. Mangrove plants sufficiently distributed in tropical and subtropical region and presently being found in the temperate part of the world. Statistics abound regarding the Niger Delta Mangrove as the largest in Africa and 5th largest globally, covering an area of over 7000sq km [18]. In the Niger Delta, mangrove plants are endemic around coastal area, deltaic swamp and Barrier Island that have affinity for high salinity, as such they are characterized as halophytes. Typically, mangroves plants grow in the intertidal zone of estuaries where they protect the water shores against hash conditions. Mangrove plants have found several applications including ecological buffers against the full impacts of ocean surge. Furthermore, most parts of the plant and their extracts have found indigenous application as curative agents for ailments [17, 19], due to their high salinity and certain unraveled bioactive metabolites [18]. Also Meenakshi and Jayaprakash [19] has stated that mangrove plants has constitutional metabolites which are biologically unparalleled. Some therapeutic compounds found in Mangrove plants include; flavonoids, triterpenes, tannins, organic acids, carbohydrates, hydrocarbons, benzoquinone, naphthofurans, sesquiterpenes, alkaloids, polymers, sulfur derivatives, and salts [17, 18].

As documented in literature, the therapeutic application of any plant is largely dependent on several enigmatic factors, which may include; location, age, organ, season and environmental stress [1], as well as the solvent used for its extraction [3, 18]. Therefore, mangrove plants represent groups of vegetation that can be used to treat water borne vectors. Studies have shown that Niger Delta mangrove plants leaves can be regarded as larvicides against mosquito [16]. Abo Zaid et al. [12] has evaluated the Molluscicidal activity of *Punica granatum, Calotropis procera*, *Solanum incanum* and *Citrullus colocynthis* against *Biomphalaria Arabica.* Hassan et al. [9] has evaluated the molluscicidal activities against snail intermediate hosts of *Schistosoma mansoni* and *Fasciola.* Hassan et al. [6] has assessed Euphorbia Aphylla, Ziziphus Spina-Christi and Enterolobium Contortisiliquum as Molluscicidal Agents. Agboola et al. [20] studied the Molluscicidal activity of fruit pericarp, leaves, seed and stem Bark of *BlighiaUnijugata* Baker. Agboola et al. [15] has studied the molluscicidal potential of some members of Nigeria Sapindaceae family. Angaye et al. [3] has studied Molluscicidal Activities of *Jatropha curcas* leaves against *Biomphalaria pfeifferi*. In a more recent study, Angaye et al. [16] investigated the larvicidal activities of Niger Delta mangrove plant against *Anopheles gambiae* (mosquito), which is endemic specie in the Niger Delta. Hence, this study aimed at assessing molluscicidal and synergicidal activities of leaves extracts of Niger Delta Mangrove plants such as *Rhizophora mangle, Rhizophora racemosa, Avicennia germinans* and *Langunculraria racemosa*.

2 Materials and Methods

2.1 Collection of plant materials

The leaves of *R. mangle*, *R. racemosa*, *A. germinas* and *L. racemosa* were collected from Femie-Ama (N4°46.7′14.8″ E7° 1.90′ 46″), and Amadi-Ama (N 4° 47.4′ 0.09″ E 7° 1.78′ 50.5″), communities of Rivers State in the month of June 2014. The voucher number of the plant was indicated and kept in the laboratory for reference purpose.

2.2 Parasite collection and bioassay

The snails of the genus *Bulinus* (*B. globosus* and *B. rholfsi*) were collected from Kanye dam in Kano State. While the genus *Biomphalaria* were collected from drainage along the Ahoda East-West road. The snails were transported to the laboratory, fed with water lettuce and bred in a plastic tank aquarium adjusted with aerator to improve oxygen supply. The applied method in this study which demonstrates the molluscicidal activity of different solvent plant extracts against the snails was developed following standard protocol [21].

2.2 Preparation of plant extracts

The leaves were shade-dried (7–8 days), at optimal environmental temperatures (29–38°C). The dried leaves were powdered mechanically using domestic electrical blender (Binatone-BLG450). Five hundred grams (500 g), of the powdered leaves was macerated in methanol (1000ml, Qualigens), for 72 hours at room temperature. Sequel to the maceration, the extract was filtered with filter paper (Whatman filter paper No. 1). The filtrate was concentrated in a rotary evaporator (60°C).

For the crude extract, fresh leaves of the plant were pounded with mortar and pestle. The juice was filtered and squeeze out using muslin cloth and concentrated in rotary evaporator (50°C), leaving no trace of liquid. The residue was recovered and stored at -4°C until it was ready for use. The positive control was set up with copper sulphate (1ppm), as per methods described by Bassey et al. [1]. On the other hand, the negative control was prepared with dechlorinated tap water (pH 7.6).

2.3 Experimental Set up/Screening

The bioassay (i.e. dose-mortality response, was setup in triplicate) was carried out following standard procedure [21], incorporating slight modification in the method, as described by several authors [1, 5, 18, 20]. Several concentrations of the plant extracts with their replicates were prepared (concentrations ranging from 50-500ppm) and tested against the snails (minimum of 20 snail within 24 hours). Extract(s), whose mortality rate was less than 100% at 500ppm after 24hours was rated as inactive [1, 18, 20], as such not applicable (N/A), for further analysis.

2.4 Statistical analysis

The median lethal dose (LC_{50}) was statistically determined using data of the average minimal mortality (ALC_{100}), from the activity screening based on Statistical package (Microsoft excel package version 2013).

3 Results and Discussion

Phytochemical results of the selected Niger Delta Mangroves plants (*R. mangle, R. racemosa, A. germinans* and *L. racemosa*) is presented in Table 1. High amounts of saponins were present in the methanolic extracts of *R. mangle and A. germinans* compared to the extracts of other species. Furthermore, alkaloid was conspicuously present in the crude extract of *A. germinans*, moderately present in the methanolic extracts of *R. mangle*, *R. racemosa*, *A. germinas* as well as the crude and methanolic extracts of *L. racemosa*. In addition, the crude extracts of *R. mangle* and *R. racemosa* indicated no trace of alkaloid. Besides the absence of glycosides in the crude extract of *L. racemosa*; result also showed that the extracts of other mangrove species had higher amount of Glycosides, Flavonoid and Tannins compared to the methanolic and crude extracts of *R. racemosa* and *L. racemosa*. The Niger Delta mangrove plants under study indicated high levels of tannins and flavonoids. Notwithstanding, the concentration of the flavonoids in the leaves may vary depending on some compounding factors, they include; salinity, species and sunlight intensity [22], as well as seasonal variation [23]. The seeds of some mangrove plant contain high level of saponin, which have found application as fish poison [24].

The phytochemical screening results from this study is comparable to previous studies. Banctaranayak [24] have reported the presence of alkaloids flavonoids, saponins, steroids, triterpenes, and tannins in some mangrove species found in Australia. Kandil et al. [25] reported that flavonoid, glycosides, tannins and other such as polyphenolics are found in the leaves of *R. mangle.* Basically, polyphenolics are known provides chemical defense against some pathogens.

Table 2 presents results of the activity screening of the four Niger Delta mangrove plants under study as well as their combined (1:1:1:1) crude and methanolic extracts. The results showed that all extracts induced varying degrees of mortalities. The crude extracts of *R. mangle* was inactive against *B. pfeifferi*, while the methanolic extracts was active against the three snails under study. The *R. racemosa* and *A. germinans* methanolic extracts was active against the three snails, while their respective crude extracts was inactive, while their respective crude extracts were inactive. The *L. racemosa* methanolic extracts against *B. globosus* and *B. rholfsi* was active against the three snails, while their respective crude extracts was inactive, while all crude extracts as well as the methanolic extract against all snails as well as the methanolic extract against *B. pfeifferi* was inactive.

Table 1: Phytochemical screening of various extracts of the different species of Mangrove plant

Species	Solvent Used	Phytochemicals Present				
		Saponin	Alkaloid	Glycosides	Flavonoids	Tannins
R. mangle	Crude Extract	+	-	++	++	++
	Methanolic Extract	++	+	++	++	++
R. racemosa	Crude Extract	+	-	++	++	++
	Methanolic Extract	+	+	+	++	+
A. Germinans	Crude Extract	+	++	++	++	++
	Methanolic Extract	++	+	+	++	++
L. racemosa	Crude Extract	+	+	-	+	++
	Methanolic Extract	+	+	+	++	+

Keys: ++ = Abundant; + = Moderate; - = Absent

Table 2: Result of activity screening for various extracts of the different species of the plant

Plant	Extracts	Vectors	Mortality Rates (%)							
			350-400 (ppm)	**300-350 (ppm)**	**250-300 (ppm)**	**200-250 (ppm)**	**150-200 (ppm)**	**100-150 (ppm)**	**50-100 (ppm)**	**0-50 (ppm)**
R. mangle	Crude Extract	*B. globosus*	100±0.00	100±0.00	100±0.00	100±0.00	91±0.85	83±1.13	77±2.031	39±1.514
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	79±1.24	73±1.09	68±0.77	51±0.64
	Methanolic Extract	*B. pfeifferi*	100±0.00	100±0.00	100±0.00	100±0.00	90±0.00	68±0.94	51±1.49	44±2.03
		B. globosus	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	89±0.33	80±0.14	69±1.43
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	93±0.73	83±1.23	77±1.44
R. racemosa	Methanolic Extract	*B. pfeifferi*	100±0.00	100±0.00	100±0.00	100±0.00	81±2.04	75±1.44	67±0.60	59±1.41
		B. globosus	100±0.00	100±0.00	100±0.00	100±0.00	96±0.10	87±.91	75±0.00	68±2.33
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	90±0.00	81±0.52
A.. germinans	Methanolic Extract	*B. pfeifferi*	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	83±1.10	76±1.40	59±2.00
		B. globosus	100±0.00	100±0.00	100±0.00	100±0.00	87±0.52	64±1.00	30±1.23	24±1.23
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	93±1.53	81±0.50	69±0.34	61±0.34
L. racemosa	Methanolic Extract	*B. globosus*	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	77±0.00	100±0.00
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	68±2.10	58±0.50
Synergy of all 4 species	Crude Extract	*B. pfeifferi*	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	81±0.37	78±1.45	71±1.43
		B. globosus	100±0.00	100±0.00	100±0.00	100±0.00	94±1.13	81±1.13	69±1.11	57±0.43
		B. rholfsi	100±0.00	100±0.00	100±0.00	91±1.04	88±1.13	77±1.13	63±1.11	51±1.11
	Methanolic Extracts	*B. pfeifferi*	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	95±0.00	89±0.00
		B. globosus	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	93±0.73
		B. rholfsi	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	88±0.73

Note: The values on the column are the mortality rates (%), with their corresponding concentrations (ppm), in the row

The median lethal dose (LC_{50}), were further evaluated statistically, as presented in Figures 1-5. The crude extracts of *R. mangle* induced activities with LC_{50} values of 165.00 and 210.06ppm against *B. globosus* and *B. rholfsi* respectively; while the methanolic extracts induced mortalities with LC50 values of 157.30, 87.50 and 108.22ppm for against *B. pfeifferi*, *B. globosus* and *B. rholfsi* respectively (Figure 1).

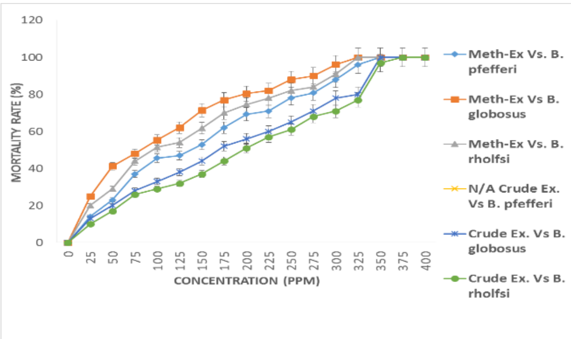

Figure 1: Dose-Mortality response for active extracts of *R. mangle*

Also, the *R. racemosa* methanolic extracts was toxic with LC_{50} values of 150.00ppm against *B. pfeifferi*, 125.00ppm against *B. globosus* and 83.51ppm against *B. rholfsi* (Figure 2). The *A. germinans*; while the *A. germinans* methanolic extracts induced mortalities of 175.00, 89.21 and 123.74ppm against *B. rholfsi, B. pfeifferi* and *B. globosus* respectively (Figure 3). Figure 4 presents results of *L. racemosa* activities. The methanolic extracts of *L. racemosa* against *B. globosus* and *B. rholfsi* was active with LC_{50} values of 102.09 and 152.03ppm respectively (Figure 4).

Compared to the individual activities of the crude and methanolic extracts, the synergicidal (combined effect of the 4 plants), formulation of the methanolic extracts against the snails demonstrated higher activities. Notwithstanding, the synergicidal crude extracts induced LC_{50} values of 237.11, 206.04 and 165.44ppm against *B. pfeifferi*, *B. globosus* and *B. rholfsi* respectively; while the synergicidal methanolic extracts were most active with LC_{50} values of 74.84, 65.10 and 28.44ppm against *B. pfeifferi*, *B. globosus* and *B. rholfsi* respectively (Figure 5).

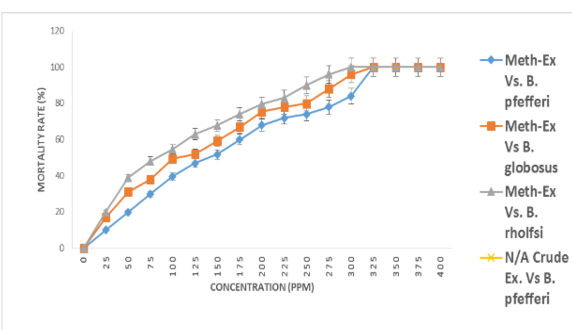

Figure 2: Dose-Mortality response for active extracts of *R. racemosa*

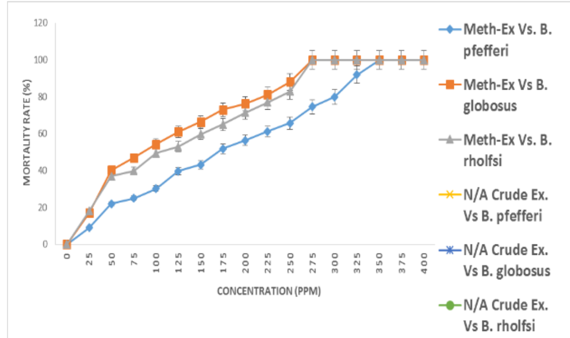

Figure 3: Dose-Mortality response for active extracts of *A. germinans*

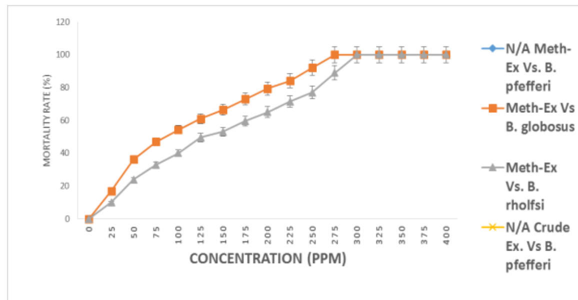

Figure 4: Dose-Mortality response for active extracts of *L. racemosa*

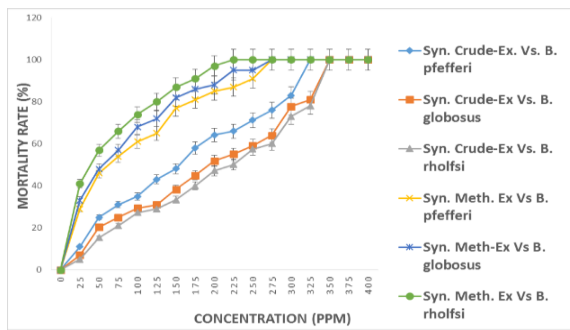

Figure 5: Dose-Mortality response for active extracts of both crude and methanolic combined formular

The results of the crude-extracts has lesser LC_{50} values compared to their respective methanolic (for *R.* mangle) and synergicidal extracts (of the four plants); as such exhibited toxic metabolites of high toxicological activities. The toxicological intensity of alcoholic extracts over crude extract had been validated in previous studies [1, 26, 7]. This could be due to the activities of the alcohol used during extraction, which has the tendency of influencing the metabolites found in the plants. However, crude extract still posses toxic metabolites (hydrophilic components), like saponins, curcin, phytates [27]. Direct application of high dosage of these extracts (i.e. crude extracts), into the environment may be lethal to other economical/eco-tolerant organisms which share same habitats (i.e. aquatic) with the intermediate host.

Several authors has reported the molluscicidal activities of plant-derived molluscucide as control agents against schistosomiasis [1, 14, 18, 20, 27]. The findings of this study has some similarity with previous reports. Agboola *et* *al.* [14] reported the molluscicidal potential of some members of Nigerian *sapindaceae* family and identified the *stem bark of Zanha goluogensis* to be highly active against *B. glabrata* snail with LC_{50} 60ppm. Hassan et al. [6] reported the molluscicidal activities of *Euphorbia aphylla* and *Ziziphus spina- chriti;* where *E. aphylla* LC_{50} value of 0.66 ppm against *Lymnae acailliaudi* and 87.6ppm against *Biomophalaria alexandrina* snails. The authors reported *Ziziphus spina- Christi* (LC_{50} = 311ppm) against *Lymnae acailliaudi* snails. Rug and Ruppel [27] used the seed of *J. curcas* against *B. natalensis* and *B. truncatus* and obtain LC_{100} values of 1ppm each; whereas in the same study, another genus *Biomphalaria* (*B. pfeifferi*) induced LC_{100} value of 25ppm. Angaye et al. [3], used 70% methanolic extract on the leaves of *J. curcas* against *B. pfeifferi* and reported mortalities with LC_{50} values of 6ppm.

The molluscicidal activities of alcoholic extracts over crude extracts of plant-derived molluscicides had already been established in literature by several authors [1, 3, 18, 20, 27]. Angaye et al. [18] reported that molluscicidal activities of *Azadirachta indica* (Neem plant) against *B. globosus* using different solvents such as chloroform, acetone, hexane and ethanol and they had LC_{50} values of 0.65, 0.45, 0.60 and 0.35ppm respectively. Angaye et al. [16] reported the larvicidal activities of *Azadirachta indica* against *Anopheles gambiae*; using hexane, chloroform and methanolic extracts and the methanolic extracts induced the highest mortalities (*R. mangle* LC_{50} = 179.38 ppm; *R. racemosa* LC_{50} = 150.00 ppm; *A. germinans* LC_{50} = 237.50 ppm; *L. racemosa* LC_{50} = 228.50 ppm). In the synergicidal use of mangrove plant against the control of mosquito, Renugadevi et al. [28] reported that the combination effect of the leaves of four mangrove plant *(Avicennia marina, Avicennia officinalis, Rhizophora apiculata* and *Rhizophora mucronata)*, was 75% more effective against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* than the combine effect of two of them (*A.marina* and *A. officinalis*), whose activities induced LC_{50} values of 34.622, 87.681 and 206.047 μg/ml respectively. Basically, mosquito larvae co-habit the same breeding habitat (water bodies), as the snail. The snails and the larvae have become major routes of the transmission for tropical vector-borne diseases (i.e. malaria and schistosomiasis), which are endemic in the Niger Delta.

In addition, human inhabitants along the coastal areas of the Niger Delta may also fall prey to the high doses of these eco-friendly molluscicides since these water bodies also are employed for a variety of uses including direct consumption, washing, bathing, transportation route etc. Plant products have over the years been appreciated over synthetic pesticides which are believed to be toxic to the bio-community of the environment to which they are applied. This study, place the mangrove plants amongst the few selected plants in the management of diseases whose vectors patronize the mollusks as their route.

Several challenges are associated with the field application of plant-derived molluscicides, including but not limited to the actual field dosage, degradability, dosage variation amongst species as well as toxicity to non-targeted/beneficial species. The molluscicidal activities of the extracts in the present study, validates previous studies, wherein the extracts from certain medicinal plants, were found to be in tandem with recent research results. The application of plant derived phytochemicals in the management of Schistosomiasis with its vector as key target is an eco-friendly control strategy because of the alteration of the line of transfer (i.e. the intermediate host).

4 Conclusions

Plants represent huge potential therapeutic resources, with over 250,000 known species currently being explored. Most plant species are endowed with rich metabolites of immense chemical diversity. Of these, only a small fraction has actually been characterized. Over the past decades, man has been exploring to understand the important values and dangers of these chemicals that are found in plant parts including when fresh, dried, fermented or processed. This study evaluated the potentials of some Niger Delta mangrove plants (*R. mangle, R. racemosa, A. germinans* and *L. racemosa*) in the control of Schistosomiasis. This study showed that these plants used in this study can be considered as putative plants for the control of Schistosomiasis using methanolic extraction.

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