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The Comparative Phytochemical and Bio-larvicidal Efficacy of Leaf Extracts of *Gmelina arborea* against Mosquito Larvae

***Tariwari C. N. Angaye[1] and Godbless N. Oyinke[2] Woyengidoubara W.T. Angaye[3], Victor D. Igbeinkutu[4]**

1-Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria
2- Department of Family Medicine, Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria.
3- Department of Internal Medicine, Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria.
4- Department of Obstetrics and Gynaecology, Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria.

Corresponding Author: Tariwari C.N Angaye, E-mail: maktarry@yahoo.com, TEL: +2347037889063

ABSTRACT
Larvicidal agents are intrinsic in the control of most vector-borne disease, due to the fact that they prevent maturity of vectors that transmit most diseases. Notwithstanding, consequent upon the fact that synthetic pesticides affect non-targeted organisms, and drug intervention could only abate morbidity burden, the application of other multifaceted approach, including bioactive plants has become desirable. The morbidity and mortality rates of malaria has become a global problem. The biolarvicidal efficacy of some solvent extracts of *Gmelina arborea* was assessed against vector of malaria (*Anopheles Gambiae*). The investigation was carried out to assess mortality rate (%), while mortality was highest in the control, all solvent extracts similarly demonstrated significant mortality rates for the monitored exposures ($p < 0.05$). Also phytochemical screening also indicated bioactive metabolites like; saponin, alkaloid, tannins, Steroid, phenol and flavonoids. Thus based on our finding, we therefore recommend *G. arborea* as potential biolarvicidal agent in the control of malaria.
Keywords: Malaria, *Gmelina arborea*, Mosquito Larvae, Vector-borne disease, Solvent extracts

1.0 INTRODUCTION

Malaria has emerged as one of the world most devastating disease, due to its significant global mortality and morbidity rate. For instance, the fact that the World Health Organization, ranked malaria topmost amongst vector-borne diseases underlines its devastating morbidity burden (WHO, 1996; Bassey et al., 2013). There are several species of mosquitoes of which only about 30 - 40 species transmit malaria in nature (Ghosh et al., 2007). However, in Africa the predominant transmitter is the female specie called *Anopheles gambiae* (Okumu et al., 2007). Statistics abounds literature globally that the disease is affecting over 3.5 billion persons annually (Ohimain et al., 2014), especially tropical Region. Report of the World Health Organization also stated that malaria is a devastating parasitic disease of global significance (Okumu et al., 2007; Aina et al., 2009). Malaria is endemic in many developing countries (especially in Africa). For instance, Okumu et al., (2007) reported that globally, there are over 100 countries, with about 700 million annual incident rate of malaria.

Generally, plants possess certain compounding metabolites which ensure their wide range of application against vector-borne diseases. For instance, some plants have been widely reported to possess repellent properties against mosquito (Egunyomi et al., 2010), vectors of lymphatic philariasis (Malebo et al., 2013), schistosomiasis (Angaye et al., 2014a), and several other pathogenic parasites (Malar et al., 2012). Notwithstanding, there are several challenges encountered in the synthetic combact against vector-borne disease. They include but not limited to the ecotoxicity of synthetic pesticides (Angaye et al., 2014a; 2014b), re-infection frequency after drug administration (Agboola et al., 2011), as well as the rapid prolificacy of the vectors (Angaye et al., 2014b).

The plant *G. arborea* is a deciduous plant which belongs to the family verbenaceae. It is most endemic in continents like Asia and Africa, including Nigeria (Offor et al., 2015). The medicinal and therapeutic application of the plant has become a mainstay in the fight against most diseases, especially in the tropical region. The antibacterial, antidiabetic and antioxidant properties of the plant have been documented in literature (Nayak et al., 2012). The root and bark extracts are believed to have found application as stomactic laxative; as well as a relief for, piles, abdominal pains and generally as an analgesic agent (Sugio et al., 2009; Saidu et al., 2012). The antimicrobial activities of the plant against some recalcitrant pathogenic organisms underline their efficacy. The plant is also believed to be effective in combating fevers and other urinary ailments (Haefliger et al. 2012).

The application of larvicides has been a practice from the foregoing, which dates back to as early as the 1890(s), although uncommon in the tropic then (Okumu et al., 2007). However, researches are currently focused on application of botanicals with against vectors in their juvenile stage (Okumu et al., 2007; Dibua et al., 2013). This strategy helps to prevent vectors from reaching maturity where they become pathogenic. Notwithstanding, problems like the toxicity of synthetic pesticides, reinfection frequency, prevalent rate in indigent endemic areas has become a source of concern. As a result, exploration of eco-friendly and effective means of control has become necessary. The objective of the research is to investigate the bio-larvicidal activities of some solvent extracts of *G. arborea* against mosquito vectors.

2.0 MATERIALS AND METHODS

2.1 Collection and preparation of plant materials

Fresh leaves of *G. arborea* were collected from Port-Harcourt City, along Eliozu-Rukpokwu road in Obio Akpor Local Government Area of Rivers State, Nigeria. The taxonomic identification of the plant was carried out using identification keys as described in literatures. The leaves were shade-dried at room temperature for 72 hours. Afterward the shade-dried leaves were placed in oven at 50^{O} C for 30 minutes. Furthermore, the leave was then powdered with an electric blender prior to solvent extraction.

2.2 Extraction Process

Three hundred grams (300 g) of the powdered leaves were macerated in 500 ml of the respective solvents being; hexane (Fisher Scientific International Company), as well as chloroform, ethanol and methanol (BHD Chemical Ltd. Poole England) for 72 hours. Meanwhile distilled water was used as solvent for the crude extraction. The filtrates of the macerated concoctions were respectively extracted using a rotary evaporator (60°C). The obtained extracts (i.e. extracted active ingredients) were allowed to cool and preserved for the bioassay at low temperature (4°C).

2.3 Vector Collection/Breeding of Larvae

Mosquito Larvae belonging to the genus *Anopheles* (*An. gambiae*), was used for this research. The larvae were cultured in the wild using baits positioned around the breeding sites (4.94889N 6.34046E; 4.93816N 6.34006E). Plastic containers and automobile tyres half-filled with water, and sand was used as the breeding bait. The baits were constantly monitored for the conspicuous emergence of larvae. Prior to the bioassay, the larvae were placed on an enamel tray with dechlorinated water (pH 7.4), and acclimatized to laboratory condition, using methods as described by Dibua et al. (2013).

2.4 Phytochemical screening of the plant extracts

Phytochemical screening of leaves of *G. arborea* for bioactive components like saponins, alkaloids, tannins, steroids, phenol, and flavonoid was carried out using protocols as described by El-Mahmood and Ameh (2007).

2.5 Experimental set up

Mosquito larvae belonging to the *A. gambiae* specie was used for the bioassay, with slight modification in a 12-hour mortality rate static non-renewal test. It was performed in consonance with WHO guidelines (WHO, 1965). A minimum 20 larvae were required per test chamber. A 500 ml solution of different concentrations of the extracts (2, 3, 4 ppm), and observed every 4 h for the mortality rate (%). Copper sulphate was used as the control.

2.6 Statistical analysis

The mean mortality and standard deviation of data from the bioassay were calculated, after which they were further subjected to concentration-mortality statistical analysis using the 2013 version of Microsoft Excel, package with 5% error.

3.0 RESULTS AND DUSCUSSION

The phytochemical screening and general biolarvicidal activities of all assayed leaf solvent extracts, including the crude, methanol, ethanol, chloroform and n-hexane extracts of *G. arborae* is presented in Table 1 and Figure 1. The result of the phytochemical analysis indicated higher concentration of saponin and alkaloid in all solvent extracts of *G. arborea*, compared to Tannin which was relatively moderate. The crude, methanol and ethanol extracts had higher concentrations of steroid compared to the chloroform and n-hexane extracts. Comparatively n-hexane extract had lower concentration of phenol compared to other extracts. While flavonoid was higher in the methanolic and ethanol extracts, the concentration of was lower in the crude extract, but conspicuously absent in the chloroform and n-hexane extracts.

Table 1. Phytochemical analysis of various leaf solvent extracts of *G. arborea*

Plants	Extracting Medium	Phytochemicals					
		Saponin	**Alkaloid**	**Tannin**	**Steroid**	**Phenol**	**Flavonoid**
G. arborea	Crude Extract	++	++	+	++	++	+
	Methanol Extract	++	++	+	++	++	++
	Ethanol Extract	++	++	+	++	++	++
	Chloroform Extract	++	++	+	+	++	-
	n-hexane Extract	++	++	+	+	+	-

++: Present in abundance; +: Present; -: Absent

An earlier study on the antimicrobial and phytochemical assessment of *G. arborea* by El-Mahmood et al., (2010), confirmed the presence of phytochemicals like; alkaloids, saponins, tannins, anthraquinones and cardiac glycosides. They also indicated that the presence of these bioactive compounds had largely supported the antimicrobial activities of the plant. In another study by Kaswale et al (2012), They unravelled secondary metabolites like; flavonoid, alkaloids, arboreal, isoarboreol, methyl arboreal, glummadiol, gmelanone, n-hexacosnol, sitostereol and hutteolin. The availability of phytochemicals is largely dependent on the applied extracting solvent (El-Mahmood et al., 2010; Kaswa et al., 2012). For instance, the absence of flavonoid in the chloroform and n-hexane extracts of G. *arborea* agrees with the findings of Kaswale et al. (2012). Phytochemical of *G. arborea* like alkaloids is believed to have stimulant with anti-malarial, and analgesic properties (El-Mahmood et al., 2010). As such the disparities of phytochemicals and their activities is largely attributed to the extraction medium or solvent.

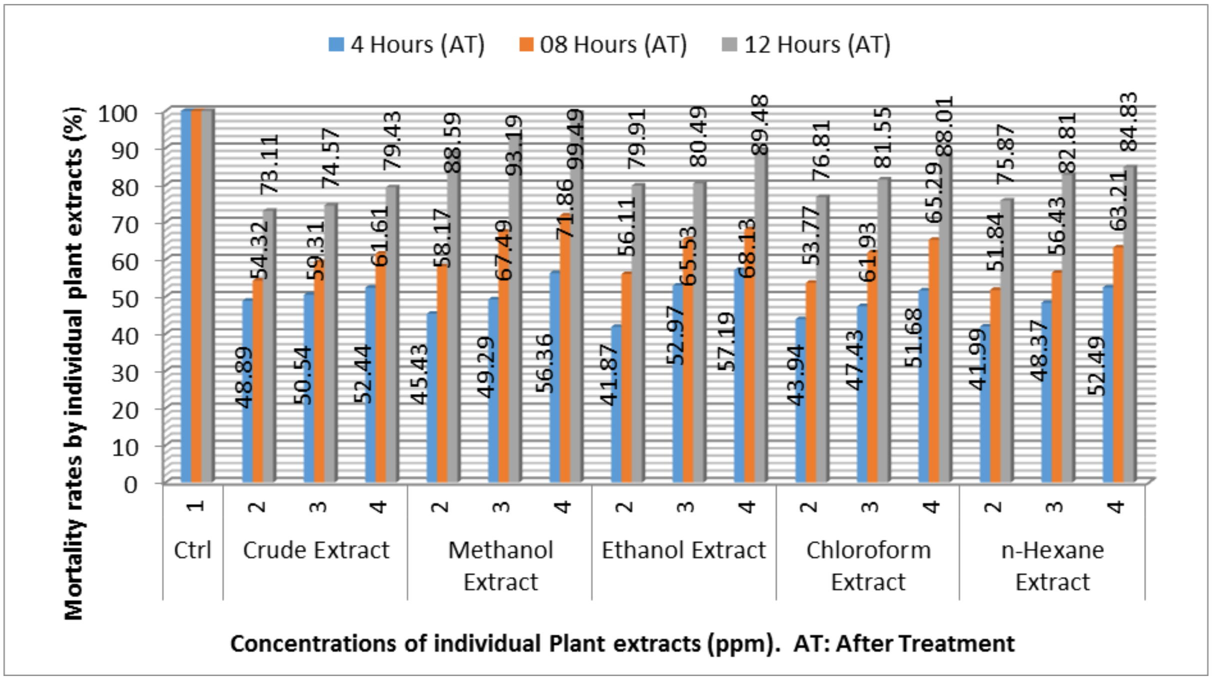

Figure 1: Mortality rates for all extracts of *G. arborea*

Figure 1 presents the average mortality rates of all solvent extracts of *G. arborea* assayed against *A. gambiae*, within exposures of 4, 8 and 12 hours at concentrations of 2, 3 and 4ppm respectively. The crude extract bioassay indicated that average mortality rates which ranged from 48.89 - 52.44% in 4 hours, in 8 hours it improved from 54.32 – 61.61%, while in 12 hours' mortality rate increased to 71.11 - 79.43%. The methanolic treatment indicated mortality rates ranging from; 45.43 - 56.36%, 58.17 – 71.86% and 88.59 – 99.94%, within exposures of 30, 60 and 90 days respectively. Furthermore, ethanol extracted G. *arborea* demonstrated mortalities within the ranges of 48.89 -56.44% in 30 days, 56.11 – 68.13% in 60 days, and 79.91 – 89.84% in 90 days. The chloroform extract was active with mortalities ranging from 43.94 – 51.68%, 53.77 – 65.29% and 76.81 – 88.01% in 30, 60 and 90 days respectively. The n-hexane treatment had mortalities of 44.99 – 52.49% (30 days), 51.84 – 63.21% (60days), and 75.87 – 84.83% (90 days). Comparatively, the control demonstrated total mortality (i.e 100% mortality), at all concentrations.

From the foregoing, the bioactive activities of *G. arborea* has been documented in literature. The antimicrobial activities of the plant were investigated against some recalcitrant pathogenic organisms like; *Escherichia coli, Klebsiella pneumonieae*, *Proteus mirabilis, Shigella dysenteriea* and *Salmonella typhi* with significant inhibitory effects (El-Mahmood et al., 2010). The most susceptible was *Salmonella typhi* (12.0 mm), while the least was, *Klebsiella pneumonieae* (7.0 mm). Furthermore, their Results also the order of activities as; chloroform (17 mm) > ethanol (13 mm) > aqueous extracts (12 mm zone diameter of inhibition) > the least was hexane extract with zone of inhibition as 9 mm. Another study using the fruit methanolic and n-hexane fruit extracts of *G. aborea* against pathogenic organisms of medical importance like; *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus morganis* had the lower inhibitory concentration of 0.001 μg for methanolic extract compared to hexane extract with 100.0 μg (Ishaku et al., 2013). The larvicidal activities of this plant indicates that it can be used in the combat against malaria, which is a major disease of concern.

CONCLUSION

Sequel to the fact that Some solvent extracts of *Gmelina arborea* demonstrated larvicidal activity against *A. gambiae* which is a vector of malaria. This indicates a therapeutic potential in strategizing for the combat against malaria. This plant could be further developed to help minimise the problems associated with chemotheraphy, which abate morbidity burden and reinfection frequency. Further studies on the plant and field trials should be encouraged in order to determine the actual efficacy of the plant as a biolarvicidal agent. Based on our findings and the broad spectrum application of the plant; we therefore also recommend the tree planting of *G. arborea* as an afforestation strategy in areas they are deemed fit.

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