Journal of Phytopathology and Pest Management 3(3): 53-62, 2016
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Essential oil from the stem bark of *Casuarina equisetifolia* exhibits mosquitocidal potential

C. Adeosun[1], I. Bamidele[1], B.Salau[1], A. Ayorinde[2*]

[1]Department of Chemical Sciences, Redeemers University, Ede Osun State, Nigeria
[2]Department of Biological Sciences, Redeemers University, Ede, Osun State, Nigeria

Abstract

Mosquitoes are insects of medical importance. They are vectors of malarial parasites, arboviruses, filarial worms amongst others. Essential oils from some botanicals have been recognized with mosquitocidal potential. Various parts of the *Casuarina equisetifolia* plants have been reported to possess antihistaminic, antioxidant, antimicrobial properties but no study on the composition of the essential oil of its stem bark and its mosquitocidal potential. The chemical composition of the essential oil from the stem bark of *Casuarina equisetifolia was* obtained by hydrodistillation and determined using gas chromatography-mass spectrometry. The mosquitocidal potential of the essential oil against *Anopheles gambiae* complex (vectors of malarial parasites) and *Aedes aegypti* (vectors of arboviruses) mosquitoes was determined by bioassay using filter papers impregnated with the crude essential oil in World Health Organization exposure tubes. The KDT_{50} and KDT_{95} were calculated by means of log time-probit analysis. Nineteen compounds were identified with the major compounds being n-hexadecanoic acid (18.67%), cis-13-octadecanoic acid (17.83%), tridecane (11.84%), Undecane (10.45%), Hentriacontane (8.91%), Nonanal (8.62%) and Oxirane (2.43%). Fatty acids were observed to have the highest relative percentage (45.43%) followed by Aliphatic hydrocarbons (37.71%). The KdT_{50} of the essential oil against *Anopheles gambiae* complex and *Aedes aegypti* was 40 minutes and 61 minutes while the KdT_{95} was 129 minutes and 212minutes against *Anopheles gambiae* complex and *Aedes aegypti* respectively. This essential oil with high percentage of fatty acids has significant mosquitocidal potential. This suggests the use of this botanical to control malaria and the arboviruses. The mosquitocidal potential of each constituent of the essential oil should be further explored.

Key words: Botanicals, essential oil, mosquito, fatty acids.

* **Corresponding author:** A. Ayorinde,
E-mail: ayorindea@run.edu.ng

Introduction

Botanical insecticides are naturally occurring compounds usually extracted from plants (Isman, 2000). Botanical insecticides are not harmful to human health as well as the environment because of their ability to decompose. (Isman, 2000). Essential oils from different plant species possess ovicidal, larvicidal and repellant properties against various insect species (Cetin et al. 2004). Botanicals insecticides include pyrethrum from crude flower dust of *Chrysanthenum cinerariaefolium* (Chandal, 2012), Rotenome from *Lonchocarpum* species (Islam, 2006) and Sabaddilla from *Schoenocaulon afficinaleI* (Zang, 1997) disrupts the insect nerve cell membrane action. Also Neem from *Azadirachta indica* (Isman et al., 1990) acts as an insect feeding constraint which can lead to the death of the insect. *Casuarina equisetifolia* of the Family *Casuarinaceae* comprises of two subspecies (*Casuarina equisetifolia spp. equisetifolia* L. and *Casuarina equisetifolia spp. incana* L) (Kikshore & Rahman, 2012). It is a nitrogen-fixing evergreen tree whose height ranges from 15 to 50m (Fig. 1). *Casuarina equisetifolia* is of Australian origin, mainly grown for its wood fuel property in Nigeria. The chemical composition of the tree includes ellagic acid, gallic acid, kaemferol and quercetin (Narayanaswamy et al. 2013). The bark possesses astringent and antioxidant properties (Narayanaswamy et al, 2013). Various parts of the plants such as the bark, leaves, seeds and fruits have been reported to possess antihistamine, antioxidant, antimicrobial, hepatoprotective and analgestic properties (Falodun 2010). The essential oil component from the leaf and fruit of *Casuarina* e*quisetifolia* has been reported (Ogunwande et al., 2011) as well as the phenolic acids present in *Casuarina equisetifolia* (Gumgumjee & Hajar, 2012). The High Performance Liquid Chromatography (HPLC) analysis revealed the plant consists of eight phenolic compounds. These include Gallic, Protocatioic, Chlorogenic, p-Hydroxybenzoic, Syringic, Vanillic and Salicyclic acid (Gumgumjee & Hajar, 2012). The phytochemical screening of *Casuarina equisetifolia* reported the presence of carbohydrates, protein, steroid and tannin in all the plant's parts. The phyto-constituents are Alkaloid (bark), Saponin (bark, fruit) and Flavonoid (in fruit and leaf) (Aher et al., 2010). The structures of condensed tannins from the stem bark and fine root of *Casuarina equisetifolia* have also been identified using MALDI-TOF MS and HPLC analyses. These contain procyanidin combined with prodelphinidin and propelargonidin with epicatechin as the main extension unit. The condensed tannins have different polymer chain lengths, varying from trimer to tridecamer for stem bark and up to pentadecamer for fine root (Shang-Ju et al., 2010). Mosquitoes affect human health in the spread of diseases such as malaria by *Anopheles* species (Woodbridge & Edward 2002) and arboviruses diseases such as yellow fever, dengue, Chikungunya infection, by *Aedes* species (Gubler, 1997). In 2015, 214 million cases of malaria were reported globally. Out of these, 35% of malaria deaths occurred in two countries- Nigeria and Democratic Republic of Congo (World Malaria Report, 2015).

The efficacy of some botanicals as insecticides has been reported. This includes leaf essential oil of *Lantana camara* (Murugesan et al., 2012) and volatile oil of *Pyrenacantha staudtii* (Falodun, 2010) on some insects. The essential oils from savory and oregano are highly effective against Indian meal moth (*Plodia interpunctella*) and Mediterrean flour moth (*Ephesha knehniella*) (Ayvaz et al., 2008). Botanicals which include plant extracts, essential oils and phytochemical have been recognized with mosquitocidal potential (Mandal, 2012). These botanicals have ovicidal and repellent activity. For instance, the leaf extracts of *Ervatamia coronaria* have repellent property against adult *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Govindarajan & Sivakumar, 2011). Also larvicidal effects of *Moriga oleifera* leaf extracts against *An. Stephensi* larvae (Prabhu et al., 2011) and repellent effect of *Eucalyptus* and A*azadirachita indica* seed oil against adult *Culex quinquefasciatus* have been reported (Mandal, 2012). The objective was to determine the chemical composition of an essential oil from the bark of *Casuarina equisetifolia* using gas chromatography (GC)- Mass spectrometry (MS) analyses and determine the potential of this plant as a mosquitocidal botanical.

Materials and methods

Plant materials: Stem bark of *Casuarina equisetifolia* was obtained from an adult tree in March 2014 from the premises of Redeemer's University, Mowe, Ogun state, Nigeria. Taxonomic identification was performed by a botanist at the Herbarium of the Botany department of the University of Lagos, Lagos, Nigeria. The bark was cleaned, dried and cut into pieces.

Hydrodistillation: The plant sample (400g) was subjected to hydrodistillation for 5 hours, using the Clevenger-type apparatus according to the European Pharmacopoeia. The volatile distillate was collected over anhydrous sodium sulfate, stored in an air-tight vial with screw lid and refrigerated at 4ºC until the time of analysis. The yield of the oil was 0.61g.

Gas chromatography-mass spectro-metry analysis: The gas chromatography (GC)- mass spectrometry (MS) analyses were realized using Agilent Technology 7890A (USA) gas chromatograph equipped with a fused silica capillary column HP-5MS (30m x 0.32m, 0.5m film thickness) on ultrapure helium gas and coupled to a mass selective detector (mass spectrometer). The injector and interface were operated at 250ºC and 380ºC, respectively. The oven temperature was raised from 60ºC to 300ºC at a heating rate of 5ºC/min and then held isothermally at that temperature. The sample was injected in a splitless mode. The MS was operated at an ionization voltage of 70eV over an acquisition mass range. The compounds were identified on the basis of their retention times and mass-spectral fragmentation patterns compared with those of reference compounds stored on the spectrometer database and National Institute of Standards and Technology (NIST) library. The constituents were percentages from the GC peak areas without any correction (Adeosun et al., 2013).

Mosquito larvae collection and rearing of Adult mosquitoes: Larvae of *Anopheles* and *Aedes* mosquito species were collected from stagnant drainages and old tires found at Redemption camp, kilometer 46 Lagos-Ibadan expressway, Ogun state, Nigeria. The larvae were reared in Redeemers University insectary to adult. The adult mosquitoes were placed in cages and fed with sugar solution (10%) soaked in cotton wool. The mosquitoes were identified into groups and species using identification keys as reported by Giles and Coetzee (Gilles & Coetzee, 1987) and Rueda (Rueda, 2004).

Bioassay on adult mosquitoes: Two to four days old non-blood fed and sugar-fed adult mosquitoes were used for the bioassay. Filter papers were impregnated with the essential oil (undiluted) from the stem bark of *Casuarina equisetifolia*. The filter papers were fixed into World Health Organization (WHO) insecticide bioassay exposure tubes. Three (3) replicates of 25 mosquitoes (*Anopheles gambiae* complex or *Aedes aegypti*) each were exposed. A control group of 25 mosquitoes (*Anopheles gambiae* complex or *Aedes aegypti*) was exposed to filter papers without essential oil from the stem bark of *Casuarina equisetifolia*. The number of mosquitoes knockdown was recorded afterr 5, 10, 15, 20, 30, 40, 50 and 60 mins. After the exposure time, the mosquitoes were transferred into holding tubes and fed with sugar solution (10%) soaked in cotton wool for 24hr.

Data analysis for the bioassay: The time taken to knock down 50% and 95% of mosquitoes (KDT_{50} and KDT_{95}) was calculated by means of log time-probit using SPSS 17.0 software.

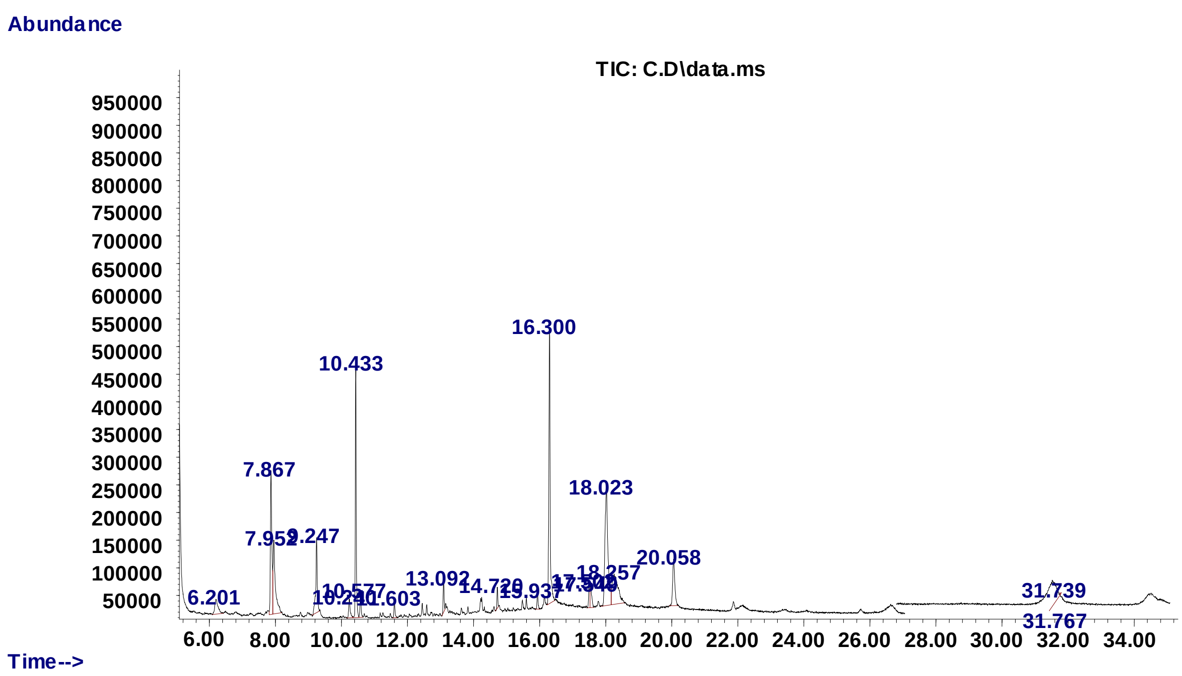

Figure 1: Gas Chromatogram of the essential oil from the bark of *Casuarina equisetifolia*.

Results

Chemical composition of *Casuarina equisetifolia* bark: Gas Chromatography (GC)- Mass Spectrum (MS) analysis on the essential oil identified and quantified nineteen constituents (100%) (Fig. 1). Quantitative composition and relative abundance of the essential oil from the stem bark of *Casuarina equisetifolia* are presented in Table 1. The main constituents were identified as n-hexadecanoic acid (18.70%), cis-13-octadecanoic acid (17.83%), tridecane (11.84%), Undecane (10.45%), Hentriacontane (8.91%), Nonanal (8.62%) and Oxirane (2.43%).Others were below 2%. The percentage of the classes of compounds found present in the essential oil is presented (Fig. 2). Fatty acids were observed to have the highest relative percentage (45.43%) followed by Aliphatic hydrocarbons (37.71%), oxygenated aliphatic hydrocarbons (13.96%), aromatic hydrocarbons (2.45%), terpenoids (2.45%) and cyclic oxygenated compounds (0.44%) (Fig. 2).

Bioassay of essential oil from the stem bark of *Casuarina equisetifolia* on *Aedes* and *Anopheles* mosquitoes: The bioassay of the essential oil from the stem bark of *Casuarina equisetifolia* on the mosquito *species* showed that it has significant adulticidal mosquitocidal activity against *Anopheles gambiae* complex and *Aedes aegypti*. The time taken to knockdown 50% (KdT_{50}) of *Anopheles gambiae* complex was 40 minutes and 61 minutes for *Aedes aegypti*. The KdT_{95} of these mosquitoes (*Anopheles gambiae* complex and *Aedes aegypti*) to the essential oil was 129 minutes and 212 minutes respectively (Table 2). The percent mortality of these two species of mosquitoes to the essential oil after 24hrs was 100%. The percentages of *Aedes aegypti* knockdown in 15, 30 and 40minutes were 1%, 10% and 15% while 14%, 25% and 26% of *Anopheles gambiae* complex were knockdown in 15, 30 and 40 minutes (Fig. 3).

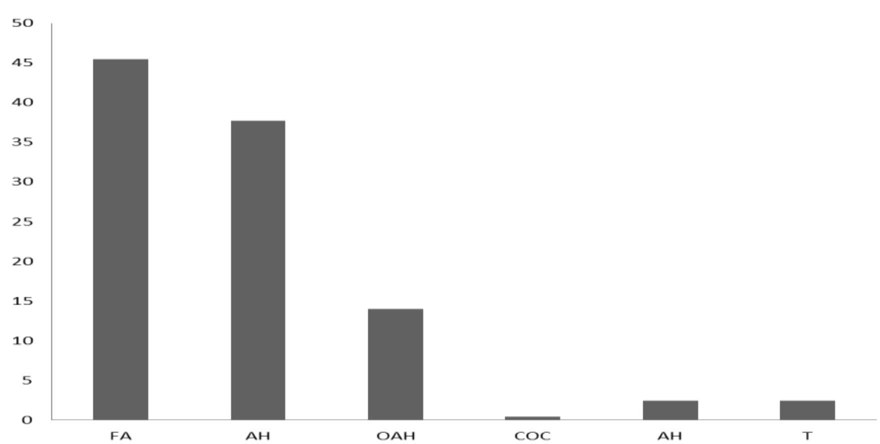

Figure 2: Relative percentages of different classes of compounds obtained from the essential oil of *Casuarina equisetifolia* stem-bark. FA= fatty acids AH = aliphatic hydrocarbons; OAH = oxygenated aliphatic Hydrocarbons; COC = cyclic oxygenated compounds; AH = aromatic hydrocarbons; T = terpenoids.

Table 1: Quanlitative and quantitative profile of essential oil obtained from the stem bark of *Casuarina equisetifolia*.

S/N	Compound	RT	% Yield
1	Oxirane	6.20	2.44
2	Undecane	7.87	10.46
3	Nonanal	7.95	8.62
4	Dodecane	9.25	5.59
5	Nonanoic acid	10.24	1.40
6	Tridecane	10.43	11.84
7	1H-indene	10.57	1.46
8	1,4-methano-1H-indene	11.60	0.94
9	Dodecanoic acid	13.09	1.50
10	Tetradecanoic acid	14.72	0.87
11	Hexadecanoic acid	15.94	0.88
12	n-hexadecanoic acid	16.30	18.67
13	9,12-octadecadienoic acid	17.50	1.41
14	9-octadecenoic acid	17.55	1.76
15	Cis-13-octadecenoic acid	18.02	17.83
16	Hentriacontane	18.26	8.91
17	Methyl ricinoleate	20.06	4.99
18	β-tocopherol	31.74	0.23
19	α-tocopherol	31.77	0.20

RT = retention time; S/N = serial number

Table 2: The KDT_{50} and KDT_{95} of the various mosquito types when exposed to the essential oil from *Casuarina equisetifolia* bark and their percentage mortality.

	% mortality (Sample size)	Susceptibility to extract	KdT_{50} (mins)	KdT_{95} (mins)
Anopheles gambiae complex.	100 (100)	Susceptible	40.20 (1.80-54.49)	129.32 (77.55)
Aedes aegypti	100 (100)	Susceptible	61.08 (53.33-73.27)	212.72 (146.09-449.13)

Discussion

The GC-MS analysis of the essential oil of *Casuarina equisetifolia* stem bark quantified nineteen constituents with fatty acids observed to have the highest relative percentage (45.43%). The major components of *Callosbruchus analis* reported to show insecticidal activity against *Desmodium elegans* were n-hexadecanoic acid, stearic acid, behenic acid, tricosanoic acid and marganic acid. The fatty acids were indicated to exhibit the insecticidal activity (Khan et al., 2012). The fatty acid-rich Turkish bryophyte extracts also reported to have insecticidal property against *Sitophilus Granarius*. The n-hexadecanoic acid, Dodecanoic acid and myristic acid were identified as the active compounds and suggested to be responsible for the adulticidal activity of the extracts (Abay et al., 2013). Fatty acid rich plants have also been reported to be responsible for insecticidal and growth inhibition activities of some pests (Farag et al., 2011). The presence of fatty acids with alkyl esters could have been responsible for the mosquitocidal potential. The synergy between fatty acids such as hexadecanoic acid and octadecanoic acid with their alkyl esters (methyl ricinoleate) promotes insectistatic and insecticidal activity (Ramos-Lopez et al., 2012). The mode of action of fatty acids depends on the insect physical contact with the fatty acid. The mode of action of lipophilic carbon chains of fatty acids in the penetration and disruption of the lipoprotein matrix of the insect's cellular membranes has been reported, (Puritch, 1981) and (Osborne & Henley, 1982). The insecticidal properties of a number of fatty acid derivatives have also been investigated (Kabara, 2001). Saturated fatty acids have optimal effects when they contain twelve carbon atoms. In this study, the saturated fatty acids had the highest proportion (62.5%) of the fatty acids of *Casuarina equisetifolia* stem bark. Furthermore, about 80% of the fatty acids of *Casuarina equisetifolia* stem bark in this study contains 12-carbon atoms, some of which are unsaturated fatty acids. The presence of the monounsaturated in the essential oil of *Casuarina equisetifolia* stem bark will increase the activity of long chain fatty acids (Kabara, 2001). The cis-unsaturated fatty acids have been reported to be more active than trans-acids. The presence of high percentage (18.02%) of cis-13-octadecanoic acid of *Casuarina equisetifolia* stem bark may be responsible for the mosquitocidal potential (Kabara, 2001). The essential oil from bark of *Casurina equisetifolia* has adulticidal activity against *Anopheles gambiae* complex and *Aedes aegypti* mosquitoes. The mortality observed after 24 hours was 100% in both *Aedes aegypti* and *Anopheles gambiae* complex. These mosquitoes are susceptible to the essential oil of *Casurina equisetifolia* stem bark. Similar reports on the mosquitocidal activity of some essential oils have been documented. The *Lantana camara* leaves against *Aedes* and *Anopheles* species also reported 93.3% and 95.2% mortalities (Dua et al., 2010). The KdT_{50} and KdT_{95} of the essential oil of *Casurina equisetifolia* stem bark were higher in *Aedes aegypti* when compared with *Anopheles gambiae* complex. The essential oil of *Casurina equisetifolia* stem bark will knockdown the *Anopheles* species faster that the *Aedes* species. Thus, it will be more effective in the control of malaria in the tropics. Some of the major components of essential oil of *Casuarina equisetifolia* stem bark identified in the present study are n-hexadecanoic acid, 9-octadecenoic acid, hexadecanoic acid and dodecanoic acid which have been reported to exhibit insecticidal properties as stated above. The reports showed that fatty acids exhibit insecticidal activity and may be suitable against several insect species. In this present investigation, essential oil obtained from the stem-bark of *Casuarina equisetifolia* showed mosquitocidal activity against adult *Anopheles* and *Aedes* mosquitoes. The present finding may be utilized for the development of plant-based pesticides as supplementary to synthetic insecticides.

References

Abay G, Altun M, Karakoc OC, Gul F, Demirtas I, 2013. Insecticidal activity of Fatty Acid-Rich Turkish bryophyte extracts against Sitophilus granaries. Combination Chemistry high Throughput Screen **16**: 806–16.

Adeosun C, Olaseinde S, Opeifa A, Atolani O, 2013. Essential oil from the stem bark of *Cordia sebestena* scavenges free radicals. Journal of Acute Medicine **3**(4): 138-41.

Aher AN, Pal SC, Yadav SK, Patil UK, Hattacharya SB, 2010. Isolation and characterization of Phyto-constituents from *Casuarina equisetifolia*. Asian Journal of Biochemistry **22**: 3429–34.

Ayvaz A, Sagdic O, Karabarklu S. Ozturk I, 2008. Insecticidal activity of the essential oils from different plants against three stored-product insects. Journal of Insect Science **10**: 1–13.

Chandal S, 2012. Mosquito vector management with botanicals the most effective weapons in controlling mosquito diseases. Asian Journal of Tropical Biomedicine **2**(4): 331.

Cetin H, Erler F. Yanikoalu A. 2004. larvicidal activity of a botanical natural product against *Culex pipens*. Fitoterapia **75**: 724–28.

Dua VK, Pandey AC Dash AP, 2010. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. Industrial Journal of Medical Research **131:** 434–39.

Falodun A, 2010. Herbal medicine in Africa, distribution and standardization. Research Journal of Phytochemistry **4:** 15–-61.

Farag M, Ahmed MH, Yousef H Abdel-Rahman AH, 2011. Repellant and insecticidal activites of *Malia Azedarach L*. against cotton leafworm *Spodoptera littoralis*. Nature Forest. **66**: 129–35.

Gilles M Coetzee M, 1987. A supplement to the Anopheline of Africa south of the Sahara. South Africa Institute of Medical Research 12–23.

. Govindarajan M, Sivakumar R, 2011. Mosquito adulticidal, and repellent activities of botanicals extracts against malaria vector, *Anopheles stephensi Liston* (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine **4:** 941–47.

Gubler, D, 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler D J, Kur G (eds). *Dengue and Hemorrhagic* fever. CAB International Washington, UK, 1-22 pp.

Gumgumjee NM, Hajar AS, 2012. Antimicrobial efficacy of *Casuarina equisetifolia* extracts against some pathogenic microorganisms. Journal of Medicine and Plants Research **6**: 5819–25.

. Islam MB, 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasing regulated world. Annual Revision of Entomology. **51:** 45–66.

Isman, M, Koul O, Luczynski A, Kaminski A, 1990. Insecticidal and antifeedent bioactivities of neem oils and their relationship to azadirachtin content. Journal of Agricultural Food Chemistry **38**: 1406–11.

Isman MB, 2000. Plant essential oils for pest and disease management. Crop Protection **19**: 603–08.

Kabara JJ, 2001. Fatty acids ans Esters as Antimicrobial or Insecticidal Agents. ACS Symposium Service **325**: 220–238.

Kikshore DV, Rahman R, 2012. Spamolytic activity of *Casuarina equisetfolia* bark extract. International Journal Pharmaceutical Research **3**: 1452–1456.

Khan A Quisar M Usman R, 2012. Fattyacid profile and Biological activities of the aerial parts of *Desmodium elegans* Dc, Middle-East Journal of Science and Research **12**: 92–95.

Mandal S, 2012. Mosquito vector management with botanicals. The most effective weapons in controlling mosquito-borne diseases. Asian Pacific Journal Tropical Biomedicine **2**: 336.

Murugesan S, Rajeshkannan C, Babu DS, Sumathi R, Manivachakam P, 2012. Identification of insecticidal properties in common weed-*Lantana Camara Linn* by gas chromatography and mass spectrum (GC.MS). Pelagia Research Library **3**: 2754-59.

Narayanaswamy V, Ninge Gowda KN, Sudhakar R, 2013. Natural dye from the bark of *Casuarina equisetifolia* for silk. International Journal of Pharmacology and Biological Science **4**: 94–104.

Ogunwande IA, Filamini G, Adefuye AE, Lawal NO, Moradeyo S, Avoseh NO, 2011. Chemical compositions of *Casuarina equisetifolia* L., *Eucalyptus tireliana* L. and *Ficus elastica roxb.exHornem* cultivated in Nigeria. South African Journal of Botany **77**: 645–49.

Osborne LS. Henley RW, 1982. Evaluation of safer Agro-Chems insecticidal soap for the control of mites in the interior environment. Foliage Digest **3**: 10–11.

Prabhu K, Murugan K, Nareshkurar A, Ramasubramanian N, Bragadeeswaran S, 2011. Larvicidal and repellent potential of *Moringa oleifera* against malaria vector, *Anopheles stephensi Liston* (Insecta: Culicidae). Asian Pacific Journal of Biomedicine **1:** 124–129.

Puritch GS, 1981. Pesticidal soaps and Adjuvants-What are they and how they work? Proceedings of the 23rd Annual lower mainland horticultural improvement association growers' short course, Abbotsford, British Columbia. Canada.

Ramos-Lopez MA, Gonzalez-Chavez MM, Cardenas-Ortega NC, Zavala-Sanchez MA, 2012. Activity of the main fatty acid components of the hexane leaf extract of *Ricinus Communis* against *Spodoptera frugiperda*. African Journal Biotechnology **11**: 4274–78.

Rueda R, 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Transmission, 1st Ed. Mangolis Press, New Zealand, 60pp.

Shang-Ju Z, Vi-Ming L, Hai-Chao Z, Shu-Dong W, Guang-Hui L, Gang-Fu V, 2010. Antioxidant tannins from stem bark and fine root of *Casuarina equisetifolia*. Molecules **15**: 5658–70.

Woodbridge AF, Edward DW, 2002. Mosquitoes (Culicidae), 203-262 pp. In Mullen G, Durden L (eds), Medical Veterinary Entomology. Academic Press, USA.

World Malaria Report, 2015. World Health Organization Malaria Program. www.who.int/malaria/visual-refresh/en

Zang X, Fukuda E, Rosien J, 1997. Method for the determination of Veratridine and carradine, major components of the natural insecticide Sabadilla, in lettuce and cucumber. Agricultural and food Chemistry **45**: 1758–61.