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Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes

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ABSTRACT

Objective: To assess the chemical composition and mosquito larvicidal potentials of essential oils of locally sourced *Pinus sylvestris* (*P. sylvestris*) and *Syzygium aromaticum* (*S. aromaticum*) against *Aedes aegypti* (*A. aegypti*) and *Culex quinquefasciatus* (*C. quinquefasciatus*).
Method: The chemical composition of the essential oils of both plants was determined using GC-MS while the larvicidal bioassay was carried out using different concentrations of the oils against the larvae of *A. aegypti* and *C. quinquefasciatus* in accordance with the standard protocol.
Results: The results as determined by GC-MS showed that oil of *S. aromaticum* has eugenol (80.5%) as its principal constituent while *P. sylvestris* has 3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl (27.1%) as its dominant constituent. Both oils achieved over 85% larval mortality within 24 h. The larvae of *A. aegypti* were more susceptible to the oils [LC_{50} (*S. aromaticum*)=92.56 mg/L, LC_{50}(*P. sylvestris*)=100.39 mg/L] than *C. quinquefasciatus* [LC_{50}(*S. aromaticum*)=124.42 mg/L; LC_{50}(*P. sylvestris*)=128.00 mg/L]. *S. aromaticum* oil was more toxic to the mosquito larvae than oil of *P. sylvestris* but the difference in lethal concentrations was insignificant (P>0.05).
Conclusion: The results justify the larvicidal potentials of both essential oils and the need to incorporate them in vector management and control.

KEYWORDS
Essential oils, Chemical analysis, Larvicides, Mosquitoes, *Pinus sylvestris*, *Syzygium aromaticum*

PEER REVIEW

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Comments
The work is novel and the article was well prepared. The methods adopted are in line with standard protocol. The results presented the chemical composition of the essential oils of two locally sourced plants, *P. sylvestris* and *S. aromaticum* and demonstrated that both plants have anti-mosquito properties which could be explored for controlling mosquito vectors towards reducing and preventing mosquito borne disease which are leading causes of wantom death and morbidity in many parts of Africa.
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1. Introduction

Mosquito borne diseases are among the leading cause of morbidity and mortality in the world[1-3]. The control of these diseases has targeted partly the reduction in mosquito populations either at developmental or adult stages[4]. The understanding was that a reduction in man-fly contact will tremendously lead to significant reduction in disease transmission and risks. Larval reduction (source reduction) has been identified as a veritable tool in mosquito control as it decimates the fly population at the stage where the insect heavily congregates and more susceptible to insecticides[5].

In the recent event of widespread of mosquito resistance to chemical insecticides, the use of plant extracts and essential oils are gaining prominence as alternative ways of controlling the insect vectors[6]. The botanicals have tremendous advantages and surmounted most of the challenges associated with chemical insecticides such

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resistance, toxicity to non-target organisms and ecological imbalances. Essential oils are volatile natural secondary metabolites characterized by a strong odour and generally have lower density than water. The oils are lipophilic in nature with proven evidence of interfering with basic metabolic, biochemical, physiological and behavioral functions of insects[7].

Syzygium aromaticum (*S. aromaticum*) is an aromatic dried flowers bud of a tree in the family Myrtaceace. *Pinus sylvestris* (*P. sylvestris*) is an evergreen coniferous tree growing up to 25 m in height and 1 m trunk diameter when mature, exceptionally to 35-45 m tall and 1.7 m trunk diameter and on very productive sites. The two oils are known to have biological activities against pathogens[8,9]. However, only limited reports exist on the insecticidal potentials of these essential oils. Yang *et al.*[8] demonstrated the potency of clove oil against *Pediculus capitis* while[10] showed that the oil of *Pinus longifolia* possesses larvicidal potential against mosquitoes. The poor socio-economic conditions of many developing countries, where access to quality health facilities is unevenly distributed necessitated the need for locally available, eco-friendly but cheap strategies to combat the menace of mosquito vectors. Most paramount among these cheap and locally available strategies is the use of plant products as mosquito repellants or larvicides.

In the present study, we assessed the chemical composition and larvicidal potentials of essential oils of two locally sourced plants, *S. aromaticum* and *P. sylvestris* against the larvae of *Aedes aegypti* (*A. aegypti*) and *Culex quinquefasciatus* (*C. quinquefasciatus*).

2. Materials and methods

2.1. Procurement of plant materials

S. aromatium buds were purchased from the local market (Oja-Oba) in Osogbo, Nigeria. *P. sylvestris* needles were obtained near Awo Hall at the University of Ibadan, Nigeria. The two plants were identified in the Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria.

2.2 Extraction of essential oils

The plant materials were subjected to hydro-distillation for 6 h in a Clevenger-type apparatus for 72 d to get enough essential oil. The essential oils of both plants were extracted using steam distillation method.

2.3 Chemical analysis of the oils

Chemical composition of *S. aromaticum* and *P. sylvestris* were analyzed by gas chromatography-mass spectroscopy (GC-MS) (Agilent 7890).

2.4 Collection of mosquito larvae

The larvae of *A. aegypti* and *C. quinquefasciatus* were collected from abandoned drums, used tyres and gutters in different parts of Osogbo metropolis, Nigeria. The larvae were transferred to the laboratory in the Department of Biological Sciences, Osun State University, Osogbo, Nigeria. The colony of the mosquitoes was maintained as previously described by Anyaele *et al*[11].

2.5 Larvicidal bioassay

The stock solution of the essential oils was prepared by emulsifying 1ml of the oil with three drops of acetone. The mixture was then made up with distilled water to make 1 litre. The working concentrations (200, 150, 120, 100, 90, 80, 50, 30 and 10 mg/L) were then prepared from the stock solution. Twenty larvae of *A. aegypti* and *C. quinquefasciatus* were exposed into 500 mL bottles containing 250 mL of each concentration. The control experiment was set up with twenty larvae containing distilled water and acetone. The bioassay was replicated four times. Larval mortality was recorded after 24 h.

2.6 Statistical analysis

Concentration-mortality lines and linear regression equation were computerized using Log-Probit analysis (Stat Plus) version 2009. The 95% confidence interval (*CI*) at the lethal concentration (LC) of 50% and 95% was obtained and subjected to chi-square to determine the differences in lethal concentrations of both oils.

3. Results

3.1 Chemical composition of the essential oils

The results of GC/MS of the two essential oils are presented in Tables 1 and 2. The oil of *S. aromatium* contains 28 compounds with Eugenol (2-Methoxy-4-(2-propenyl)phenol) (80.5%) and Eugenyl acetate (4-Allyl-2-methoxyphenyl acetate) (5.01%) constituting the major constituents. The oil of *P. sylvestris* is made up of 30 compounds. The oil has 3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl, 3-Cyclohexen-1-ol, 1 methyl-4-(1-methylethyl), Cyclohexanol, 1-methyl-4-(1-methylethenyl) as principal constituents.

3.2 Larvicidal properties of the essential oils

The larvicidal efficacies of the oils against the 4th instar larvae of *A. aegypti* and *C. quinquefasciatus* showed that the mortality is dose dependent; the mortality increases as concentration increases (Tables 3 and 4). Both oils achieved 98.33% larval mortality against *A. aegypti*. The LC_{50} and LC_{95} varied but the difference was not significant (P>0.05). The oil

Table 1

Chemical composition of *S. aromaticum*.

Compound present	Retention time	% COMP.
α-thujene (Bicyclo[3.1.0]hept-2-ene, 4- methyl-1-propan-2-yl)	4.014	0.26
α-pinene (Bicyclo[3.1.1]pent-2-ene,1S,5S-2, 6,6-Trimethyl-)	4.403	0.48
Camphene(Bicyclo[2.2.1]heptane 2,2-dimethyl-3-methylene-)	4.649	0.70
Oleic acid	4.975	0.56
Myrcene(1,6-octadiene,7-Methyl-3-methylene-)	5.444	1.84
α-phellandrene (2-Methyl-5-(1-methylethyl)-1,3-cyclohexadiene)	5.685	0.49
α-terpinene(cyclohexa-1,3-diene, 1-methyl-4-propan-2-yl-)	7.212	1.65
p-cymene(Benzene,1-Methyl -4-(1-methylethyl)-)	7.779	0.47
Limonene (cyclohexene,1-methyl-4-(1-methylethenyl)-)	8.391	0.46
Octadecanoic acid	8.494	0.44
Trans-sabinene hydrate (Bicyclo[3.1.0]hexane 4-methylene-1-(1-methylethyl)-)	8.963	0.13
Trans-β-ocimene(1,3,6-octatriene,3,7-dimethyl-)	9.043	0.17
linalool (octa-1,6-dien-3-ol,3,7-dimethyl-)	10.228	0.12
Terpinen-4-ol (cyclohexen-4-ol ,1-methyl-4-isopropyl-1-)	10.743	0.91
α-terpineol (propan- 2-ol ,2-(4-Methyl- 1-cyclohex- 3-enyl)-)	11.000	0.06
Thymol (phenol,2-Isopropyl-5-methyl-)	11.155	0.44
Eugenol (2-Methoxy-4-(2-propenyl)phenol)	11.429	80.95
α-copaene (tricyclo[4.4.0.0]dec-3-ene (1*R*,2*S*,6*S*,7*S*,8*S*)-8-isopropyl-1,3-dimethyl)-)	11.939	0.50
Caryophyllene oxide((1R,4R,6R,10S)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.0]4,6)]dodecane)	12.116	0.10
cis-Z-.alpha.-Bisabolene epoxide	12.271	0.05
β-caryophyllène ([7.2.0]undec-4-ene,(E)-caryophyllene)	12.232	3.14
α-humulene (1,4,8-cycloundecatriene,2,6,6,9-Tetramethyl-)	12.625	0.28
Tricyclo[3.2.2.0]nonane-2-carboxyl ic acid	13.283	0.07
Terpinolene(4-methylene-1-(1-methylethyl)cyclohex-1-ene)	13.466	0.60
δ-cadinene ((1S,4aR,8aR)-4,7-dimethyl-1-(propan-2-yl)- 1,2,4a,5,6,8a-hexahydronaphthalene (α-cadinene))	14.290	0.20
Eugenyl acetate (4-Allyl-2-methoxyphenyl acetate)	17.689	5.01
β-pinene (6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane)	19.738	0.14
β-phellandrene(3-Methylene-6-(1-methylethyl)cyclohexene)	19.824	0.46

Table 2

Chemical composition of *P. sylvestris*.

Compounds present	Retention time	% COMP.
(+)-4-Carene	3.201	0.21
Fenchol	3.573	1.74
Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	3.728	3.52
3-Cyclohexen-1-ol, 1 methyl-4-(1-methylethyl)	4.346	21.82
Cyclohexanol, 1-methyl-4-(1-methylethenyl)	4.597	14.07
Cyclohexanol, 1-methyl-4-(1-methylethenyl)	4.700	3.83
Isoborneol	4.786	2.55
Borneol	5.021	6.72
3-Cyclohexene-1-methanol, .alpha., .alpha.4-trimethyl	5.753	27.17
Ethanone, 1-(2,5-dihydroxyphenyl)	6.365	0.09
Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl	7.252	0.51
Benzene, 1-methoxy-4-(1-propenyl)	7.338	0.20
(+)-Cycloisosativene	7.504	0.21
Longifolene-(V4)	7.613	0.42
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-m ethylethyl)-, (1α, 4a.α, 8a.α.)	7.985	0.23
Terpin Hydrate	8.185	0.52
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-Methylene-, [1.alpha. 3a.beta,4.alpha, 8a.beta.)]	8.465	5.45
Caryophyllene	8.700	1.30
α-Caryophyllene	9.301	0.16
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)	9.404	0.16
Heptafluorobutanoic acid, 2-(1-adamantyl)ethyl ester	16.728	0.14
Benzenemethanol, .alpha.-ethyl-.al pha.-2,5,7-octatrienyl	16.877	0.47
Dichloroacetic acid, 1-adamantylmethyl ester	17.111	0.17
3-Adamantanecarboxylic acid, phenyl ester	17.214	0.20
n-Hexadecanoic acid	17.489	1.32
trans-13-Octadecenoic acid	19.703	4.71
Hexadecanoic acid, 2-hydroxy-, methyl ester	19.795	1.19
Cholesta-3,5-diene	25.929	0.15
Benzene, 1,3-bis(3-phenoxyphenoxy)	33.831	0.21
Oleic acid	34.649	0.57

of *S. aromatium* had LC_{50} of 92.56 mg/L and LC_{95} of 137.80 mg/L while *P. sylvestris* had the LC_{50} and LC_{95} of 100.39 mg/L and 142 mg/L respectively.

Table 3

Larvicidal activity of *S. aromaticum* and *P. sylvestris* oils against *A. aegypti*.

Concentration (mg/L)	Mortality of *S. aromaticum* (%)	Mortality of *P. sylvestris* (%)
30	1.67	1.67
50	6.67	1.67
80	13.33	13.33
90	20.00	13.33
100	86.67	73.33
120	86.67	80.00
150	98.33	93.33
Control	0.00	0.00
LC_{50} (mg/L)	92.56	100.39
LC_{95} (mg/L)	137.80	142.29

Table 4

Larvicidal activity of *S. aromaticum* and *P. sylvestris* oils against *C. quinquefasciatus*.

Concentration (mg/L)	Mortality of *S. aromaticum* (%)	Mortality of *P. sylvestris* (%)
30	1.67	1.67
50	1.67	1.67
80	6.67	6.67
90	6.67	6.67
100	13.33	13.33
120	20.00	13.33
150	93.33	86.67
Control	0.00	0.00
LC_{50} (mg/L)	124.42	128.19
LC_{95} (mg/L)	173.82	183.40

There was variation in larval mortality of both oils against *C. quinquefasciatus*. The oil of *S. aromatium* achieved 93.33% larval mortality while *P. sylvestris* recorded 86.67%. The LC_{50} and LC_{95} of *S. aromaticum* were 124.42 mg/L and 173.82 mg/L respectively while *P. sylvestris* recorded 128.19 mg/L and 183.40 mg/L as LC_{50} and LC_{95} respectively. The difference in lethal concentrations of both oils was not statistically significant (P>0.05).

4. Discussion

The promising larvicidal potentials of some essential oils against mosquito vectors[6,7,12] have given impetus to explore the possibility of using essential oil-based products as supplementary and complimentary measures for mosquito borne diseases. Chemical elucidation of the essential oils of *S. aromaticum* and *P. sylvestris* locally sourced from Southwestern Nigeria revealed that the constituents compared with previous reports on the oils[10,13-15] but with slight variations. The variations in the composition may be associated with chemotypes for the same or different species or as a result of environmental, developmental and physiological differences[10,15].

The results of the larvicidal efficacies of the two oils showed that both plants are highly toxic to the mosquito larvae as both achieved over 85% larval mortality within 24 hours and there was no significant difference in lethal doses of both plants. Both plants have been reported to have high biological activities against pathogens and insect pests[8,10]. However, the oil of *S. aromaticum* was more toxic to the larvae than *P. sylvestris*. The difference in toxicity of the oils may plausibly be due to the variation chemical composition of the oils which would have determined the bioactivity of the plant against the mosquito larvae[10,15]. *S. aromaticum* has eugenol (80.5%) as its principal constituent which has been reported to exhibit high insecticidal and antimicrobial properties and it has been incorporated in many formulations to control insect pests and pathogens[9].

The larvae of *A. aegypti* were more susceptible to the oils than the larvae of *C. quinquefasciatus*. The varying level of susceptibility of insect vectors to insecticides have been known to be associated with many factors among which are physiological, and biochemical differences of the insects[10,16]. Earlier authors have also higher susceptibility of *A. aegypti* to insecticides than *C. quinquefasciatus*[10]. This observation hitherto justifies the findings of this study.

In conclusion, the findings of this study demonstrate the larvicidal potentials of the essential oils of *P. sylvestris* and *S. aromatium* against the common mosquito vectors. These locally sourced essential oils can therefore be incorporated in the mosquito control measures, mostly in local areas where access to health facilities is extremely difficult.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The background of the research is alright as relevant and recent literature were used. The work is of immense importance in the recent quest to do away with the chemical control but pursue a cheap and an eco-friendly approach to deal with the menace of mosquito borne diseases. However, the authors should elicit interest in the work by coming up with more strong reasons for the work.

Research frontiers

The article is novel as it elucidates the chemical composition of the essential oils of two locally sourced plants, *P. sylvestris* and *S. aromaticum* using high tech equipment. It also looked at alternatives that are cheap and readily available for the control of mosquitoes. This is especially relevant in African local settings where lack of power supply and poor status of the people do not allow them to have access to health facilities. Thus, the use of local remedies to combat the menace of the mosquito vectors becomes imperative and this is emphasized in this study.

Related reports

The Materials and Methods section is well written and concise. The methods adopted are standard method in vector control. The authors reported that the larvae of *A. aegypti* were more susceptible to the oils of *P. sylvestris* and *S. aromaticum* than the larvae of *C. quinquefasciatus*. The study further justified that the varying level of susceptibility of insect vectors to insecticides have been reported in the literature. The chemical composition of essential oils of *P. sylvestris* and *S. aromaticum* however compared with other species in the same family of the plants but with some variations.

Innovations and breakthroughs

The paper reported the chemical composition and larvicidal potentials of two locally sourced plants, *P. sylvestris* and *S. aromaticum*. According to the authors, these plants are commonly found in many parts of Southwestern Nigeria but their potentials as larvicides are relatively scanty in the literature. This study however reports the larvicidal potentials of these plants.

Applications

The paper has wide application as it has contributed to knowledge in the area of ethono-botanical, characterization of chemical properties of plants and vector control, mostly in rural settings where poor socio-economic conditions may hinder access to health facilities and protection against insect vectors using modern techniques. Plant larvicides have been known to be eco-friendly and very cheap to procure. The incorporation of the essential oils of the two plants, *P. sylvestris* and *S. aromaticum*, these plants in integrated vector control would go a long way to reduce the population of mosquitoes in the environment.

Peer review

The work is novel and the article was well prepared. The methods adopted are in line with standard protocol. The results presented the chemical composition of the essential oils of two locally sourced plants, *P. sylvestris* and *S. aromaticum* and demonstrated that both plants have anti-mosquito properties which could be explored for controlling mosquito vectors towards reducing and preventing mosquito borne disease which are leading causes of death and morbidity in many parts of Africa.

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