

Larvicidal Potential of *Commiphora swynnertonii* (Burtt) Stem Bark Extracts against *Anopheles gambiae* ss, *Culex quinquefasciatus* Say and *Aedes aegypti* L

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Abstract: *Petroleum ether, ethyl acetate and methanol stem bark extracts of Commiphora swynnertonii were evaluated for larvicidal potential against laboratory reared late third stage instar of mosquito namely, Anopheles gambiae ss Gile, Culex quinquefasciatus Say and Aedes aegypti L. The WHO methodology was adopted with minor modification using methanol extract with concentrations ranged from 25-300µg/mL and ethyl acetate and petroleum ether extracts with concentrations ranged from 5-50µg/mL. The activity was time and dose dependent where, ethyl acetate extract revealed higher larvicidal activity with LC₅₀ ranged from 14.6395-3.9455µg/mL, 25.1096-5.3442 µg/mL, 27.0405-8.4829 µg/mL for Aedes aegypti, Culex quinquefasciatus, and Anopheles gambiae at 24h, 48h, and 72h of exposure respectively. Among the three species of mosquito larvae tested, Anopheles gambiae was found to be relatively resistant to extracts followed by Culex quinquefasciatus and the weakest was Aedes aegypti. These results validate use of Commiphora swynnertonii as a potential botanical larvicidal agent in controlling mosquitoes and the spread of mosquito borne diseases.*

Keywords: *Anopheles gambiae, Culex quinquefasciatus, Aedes aegypti*, larvicidal activity, Lethal concentration.

1. Introduction

Mosquitoes are intermediate and vector of several diseases of animals and humans importance. The people who are at higher risk of mosquito borne diseases are from tropical countries and the mostly affected ones, are developing countries (Snow et al., 2005). In Tanzania, malaria is one of the most important causes of direct or indirect infant, pregnant mothers and adult mortality (Mboera et al., 2007), over 80% of the country and about 20% of the population live in unstable malaria transmission areas prone to malaria epidemics (NMCP, 2008). Furthermore, the number of clinical malaria cases per year is estimated to be 14 – 19 million resulting in 100,000 and 125,000 deaths with approximately 80,000 deaths for children fewer than five years of age (WHO, 2009). Over 40% of all outpatient attendances are attributable to malaria (NMCP, 2008). According to the 2004 update of the Global Burden of Diseases (GBD), malaria leads by 20%, and neglected tropical diseases accounted for 6%, half of which is associated to lymphatic filariasis (LF) which is a major cause of permanent and long-term disability to humans (Bockarie et al., 2009; Muturi et al., 2008). Filariasis is endemic in all regions of Tanzania mainland and Zanzibar islands, with higher susceptibility levels ranged 45–60% observed along the coast, and lower levels in the western portion of the country (Castro et al., 2010).

The female mosquitoes which are vector for various diseases are involved in feeding on human blood and responsible for the transmission of a number of diseases (Malebo et al., 2013). The disease caused by mosquitoes includes human and avian malaria, human and animals filariasis, rickettsial

infections and viral infections of man and animals including Rift valley fever, Yellow fever, Chikungunya, Eastern and Western Equine encephalitis, West Nile fever, dengue fever, St Louis and Japanese B encephalitis (Goma, 1966; Kabula and Kilonzo, 2005). Among these mosquito borne diseases dengue fever, dengue hemorrhagic fever, yellow fever and chikungunya, are transmitted by *Aedes aegypti* L (Shivakumar et al., 2013), Malaria is transmitted by *Anopheles* species, one of it namely *Anopheles gambiae* (Maharaj et al., 2012) and filariasis is transmitted by *Culex quinquefasciatus* (Kabula and Kilonzo, 2005). Other mosquito borne diseases such as West Nile fever, St Louis and Japanese B encephalitis are transmitted from birds to man and other mammals by infected mosquitoes of *Culex* species. These diseases not only cause high levels of morbidity and mortality, but also cause great economic loss and social disruption on developing countries such as Tanzania.

The use of synthetic insecticides has been very effective in reducing mosquito borne diseases transmission, however, over time success has been challenged by the development of insecticide resistance (Maharaj et al., 2011). For example, DDT was among the insecticide used to control malaria in Zanzibar but reported resistance to *Anopheles gambiae* after some years of use (Prapanthadara et al., 1995) likewise to *Anopheles taeniorhynchus* (Raja et al., 2014). The development of resistance, undesirable effects on non-target organisms have made conventional chemical insecticides to create environmental and human health concerns (Chowdhury et al., 2008). Because of resistance in the vectors, most of classes of synthetic insecticides have become ineffective (Vinayagam et al., 2008); (Vincent,

2000). These problems have necessitated much investigation to botanicals which have shown great success in hindering growth and multiplication of insects to replace synthetic insecticides (Nyamoita et al., 2013). The variety of plants are reported to contain insecticidal compounds, such as saponine, steroids, isoflavonoids, essential oils, alkaloids and tannins, with higher activity in disrupting larvae survival as well as adult vectors (Ghosh et al., 2008; Joseph et al., 2004; Cavalcanti et al., 2004; Khanna and Kannabiran, 2007). Furthermore, botanicals were found to offer an advantage over synthetic insecticides, as they are less toxic, less prone to the development of resistance and easily biodegradable (Kabula and Kilonzo, 2005). Therefore, medicinal plants are very effective in mosquito control because plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitoes (Yang et al., 2004; Maharaj et al., 2010).

The control of mosquitoes using mosquito nets impregnated with natural insecticides, and the use of plant repellents is highly recommended to reducing mosquito bites (Nyamoita et al., 2013; Malima et al.; Wanzala and Ogoma, 2013; Malebo et al., 2005). Mosquitoes in the larval stage are attractive targets for insecticides because they breed in water, easy to treat in majority as they are less mobile compared to adult mosquitoes and thus, are easy to deal with them in this habitat (Chowdhury et al., 2008; Ghosh et al., 2012). Therefore, larval control is effective to reduce mosquito borne diseases transmission both in rural and urban settings.

This study screen for larvicidal potential of stem bark extracts of a medicinal plant *Commiphora swynnertonii* so as to validate use, safety and efficacy against named mosquitoes

2. Material and Method

2.1 Experimental Site

The extraction process was done at the Institute of Traditional Medicine, Muhimbili College of Health Sciences and larvicidal activity was done at the Nelson Mandela Institution of Science and Technology.

2.2 Plant Material Collection

The stem bark was collected from plants *Commiphora swynnertonii* growing in their natural environment at Manyire Village in Meru district Arusha. Identification of a plant was done by Mr. Haji Selemani a botanist from the Department of Botany, University of Dar es Salaam and voucher specimen number CS 6872 is deposited in the herbarium at the Nelson Mandela African Institution of Science and Technology.

2.3 Chemical reagents and media

Dimethyl sulphoxide (DMSO) was obtained from RFCL Limited (Haryana-India). Analytical solvents were brought from RFCL Limited (Haryana-India). Distilled water was obtained from the Nelson Mandela African Institution of Science and Technology distiller.

2.4 Plant Material Processing

The collected stem bark was air dried at room temperature. After dryness the stem bark was pulverized into powder to provide larger surface area for solvent to dissolve the compounds.

2.5 Extraction

The sequential extraction was done using solvents in the order of increasing their polarity namely; petroleum ether, ethyl acetate, and methanol respectively. The powdered plant materials (1000g) were soaked in the extracting solvents for 24h. The extract was filtered through a Whatman No. 1 filter paper, and then concentrated *in vacuo* using Rotary evaporator. The obtained extracts were kept at 4°C until further use.

2.6 Larvicidal Potential Assay

The larvicidal test was performed according to World Health Organisation (WHO) protocol with minor modification. The stock solutions (100 mg/mL) of stem bark extract were prepared by first dissolving them in DMSO. The dilution of stock solutions was made with distilled water to make 100 mL of 300, 200, 100, 50 and 25 µg/mL solutions of methanol extract and 50, 25, 15, 10, and 5 µg/mL solution of ethyl acetate and petroleum ether extracts. Ten late third instar laboratory reared *Anopheles gambiae*, *Culex quinquefasciatus*, and *Aedes aegypti* mosquito larvae were then introduced in the test solutions and mortality was observed after 24 h, 48 h and 72 h. Negative control tests contained mosquito larvae, DMSO (0.5%) and water only. All tests were carried out in triplicate under controlled temperature (26 ± 2°C) and relative humidity of 75-85%. The number of dead larvae was recorded after 24 h, 48 h, and 72 h, and the mean percentage mortalities were calculated for each concentration. The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program. The concentrations killing fifty percent of the larvae (LC₅₀) were calculated from the regression equations obtained from the graphs.

3. Results

The results demonstrated higher larvicidal activity from ethyl acetate extracts to all mosquito larvae tested namely, *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti* (Table 1, 2 and 3) According to (Komalamisra et al., 2005; Bucker et al., 2013), classification of plant larvicidal activities is considered as nontoxic when the LC₅₀ is greater than 750 µg/mL, weakly effective (LC₅₀ is between 200 to 750 µg/mL), moderate (LC₅₀ is between 100 to 200 µg/mL), effective (LC₅₀ is between 50 to 100 µg/mL), and highly effective (LC₅₀ is less than 50 µg/mL). Therefore ethyl acetate preceded in activity and petroleum ether followed and the least was methanol extract despite of higher concentrations used which ranged from 25-300 µg/mL. Thus, medium polar secondary metabolites exhibited higher larvicidal potential than polar and non polar compounds.

According to Komalamisra et al., 2005 the extracts which showed no activity after 24h of exposure was methanol extract with LC₅₀ 1235.6784 and 828.1259 µg/mL for *Aedes aegypti* and *Culex quinquefasciatus* respectively (Table 3 and 2), but the dose and time dependent trends have made methanol extract to be efficient to moderate in activity over

Aedes aegypti, *Culex quinquefasciatus* and *Anopheles gambiae* with LC₅₀ 26.5528, 86.5375 and 238.3535 µg/mL respectively after 72 hours of exposure (Table 1,2,and 3).

Table 1: Larvicidal activity of *Commiphora swynnertonii* against *Anopheles gambiae*

Extracts	Time(h)	LC ₅₀ (µg/mL)	95% C.I (µg/mL)	R ²	Regression equation
CSSP	24	485.457	248.875-946.9324	0.9265	Y=26.5logx-21.183
	48	28.8653	20.9062-39.8543	0.9691	Y=54.879logx-30.144
	72	9.9192	7.9012-12.4525	0.9696	Y=77.832logx-27.558
CSSE	24	27.0405	15.7744-46.3528	0.8815	Y=50.489logx-22.301
	48	11.7838	8.9747-15.4721	0.8763	Y=65.017logx-19.652
	72	8.4829	6.5313-11.0175	0.8987	Y=67.725logx-12.886
CSSM	24	709.3404	480.29-1047.6248	0.8118	Y= 58.614logx-117.1
	48	307.8572	216.4959-437.7729	0.7909	Y=56.22logx-89.895
	72	238.3535	123.3394-460.6181	0.7145	Y=30.046logx-21.426
Control (-ve)	NM	-	-	-	-

Key: CSSP-*Commiphora swynnertonii* petroleum ether extract, CSSE-*Commiphora swynnertonii* ethyl acetate extract, CSSM-*Commiphora swynnertonii* methanol extract, LC₅₀- Lethal concentration (concentration to kill 50% of test organisms), C.I-Confidence Interval, R²-Regression coefficient

Table 2: Larvicidal activity of *Commiphora swynnertonii* against *Culex quinquefasciatus*

Extracts	Time (h)	LC50 (µg/mL)	95% C.I	R2	Regression equation
CSSP	24	27.8146	7.5569-102.2409	0.982	Y=13.6021logx+30.36
	48	13.7641	4.9862-39.9944	0.9518	Y=19.503logx+27.79
	72	3.0514	1.5963-5.8327	0.9768	Y=27.331logx+36.758
CSSE	24	25.1096	15.9345-39.5677	0.900	Y=38.931logx-4.4972
	48	8.9015	6.0856-13.0202	0.9836	Y=46.556logx+5.797
	72	5.3442	3.6199-7.8896	0.9904	Y=45.448logx+16.92
CSSM	24	828.126	435.6952-1574.0188	0.9449	Y=27.569logx-30.449
	48	297.087	90.2561-977.8918	0.9797	Y=14.862logx+13.248
	72	86.5375	29.8796-250.6299	0.793	Y=18.616logx+13.937
Control(-ve)	NM	-	-	-	-

Key: CSSP-*C. swynnertonii* petroleum ether extract, CSSE-*C. swynnertonii* ethyl acetate extract, CSSM-*C. swynnertonii* methanol extract, LC₅₀- Lethal concentration (concentration to kill 50% of test organisms) C.I-Confidence Interval, R²-Regression coefficient, NM-No mortality

The activity revealed by ethyl acetate to *A. gambiae* (LC₅₀ 27.0405 µg/mL) corroborate with Table 2 and 3 for *Culex quinquefasciatus* and *Aedes aegypti* with LC₅₀ 25.1096 µg/mL and 14.6392 µg/mL respectively. Therefore, from this

study, secondary metabolites responsible for higher larvicidal activity are from ethyl acetate extracts of *Commiphora swynnertonii* stem bark.

Table 3: Larvicidal activity of *Commiphora swynnertonii* against *Aedes aegypti*

Plant extract	Time of exposure(h)	LC50 (µg/mL)	95% C.I	R2	Regression equation
CSSP	24	24.9940	15.3601-40.6702	0.9913	Y=36.366logx-0.8337
	48	3.5402	2.0220-6.1981	0.9416	Y=31.614logx+32.643
	72	1.6390	0.8935-3.0062	0.9624	Y=29.191logx+43.736
CSSE	24	14.6395	11.3440-18.8922	0.9321	Y=69.414logx-30.904
	48	5.8130	4.2218-8.0039	0.9494	Y=55.357logx+7.685
	72	3.9455	2.7027-5.7596	0.9504	Y=46.805logx+22.099
CSSM	24	1235.678	666.0620-2292.431	0.9328	Y=28.649logx-38.58
	48	120.9375	61.2620-238.7427	0.9701	Y=26.034logx-4.2174
	72	26.5528	14.3226-49.2262	0.8602	Y=28.683logx+9.1522
Control (-ve)	NM	-	-	-	-

Key: CSSP-*C. swynnertonii* petroleum ether extract, CSSE-*C. swynnertonii* ethyl acetate extract, CSSM-*C. swynnertonii* methanol extract, LC₅₀- Lethal concentration (concentration to kill 50% of test organisms) C.I-Confidence Interval, R²-Regression coefficient, NM-No mortality

4. Discussion

The highest larvicidal activity demonstrated by ethyl acetate extracts, indicated medium polar secondary metabolites are responsible for the activity. The effectiveness of ethyl acetate extracts were seen to all larvae tested including *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti* with LC₅₀ 27.0405, 25.1096, and 14.6395 µg/mL respectively (Table 1, 2 and 3). These results are comparable to *Commiphora caudate* ethyl acetate extracts which showed significantly higher larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* (Baranitharan and Dhanasekaran, 2014). In general percentage mortality of all species of larvae to methanolic extracts were relatively low, thus higher LC₅₀ (Table 1, 2, and 3) as compared to petroleum ether and ethyl acetate extracts. However methanolic extract exhibited unsubstantial activity to *A. aegypti* with LC₅₀ 1235.678 µg/mL (Table 3) also ineffective to *A. gambiae* and *C. quinquefasciatus* with LC₅₀ 709.3404 and 828.126 µg/mL (Table 1 and 2). These results indicate that methanol extract of *Commiphora swynnertonii* was non-toxic at first 24h of exposure to larvae. From *Sterculia quinqueloba* methanolic extract displayed weak activity for both *A. aegypti* and *C. quinquefasciatus* after 72h of exposure with LC₅₀ value range from 200 - 750µg/ml (Wilson et al., 2014) and the *Anopheles gambiae* was the stronger at inhibiting activity of extract with LC₅₀ 3662.4 µg/mL. Furthermore, from this study *A. gambiae* was seen to be relatively resistant to extracts followed by *C. quinquefasciatus* and the least is *A. aegypti* in the first 24hours of exposure, but other trends of activity changed as per time and dose dependent (Tables 1,2, and 3). The study done by Habeeb et al., (2009) exhibit larvicidal potential from *Commiphora molmol* and *Allium cepa* with LC₅₀ 0.992 and 0.383 respectively against *Culex pipiens*, and the displayed toxicity was due to secondary metabolites which are 1, 8-Cineole 12.11%, l-linalool 43.36% and Camphor 0.17% for *Allium cepa* and dl-limonene 12.25% for *Commiphora molmol*. The physiological changes of larvae due to *Commiphora molmol* extracts revealed inhibitory action over protein contents of larvae, thus larvicidal activity of the oleo-resin and oil was explained to be related to the loss of certain enzymes inhibited by these extracts which affect the metabolic processes (Massoud et al., 2001), moreover histological examinations of Myrrh treated mosquito larvae showed great pathological effect on their fat, muscles, gut and nervous tissues (Massoud et al., 2000)

5. Conclusion

The study publicized ethyl acetate extract of *Commiphora swynnertonii* to have higher larvicidal activity compared to petroleum ether and methanol extracts. Further study has to be done to isolate pure compound that will exhibit higher larvicidal activity from *Commiphora swynnertonii* extracts.

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