

# Brine shrimp cytotoxicity, phytochemical screening and larvicidal activities of *Plectranthus barbatus* extracts

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## Abstract

The genus *Plectranthus* (Lamiaceae) represents a large and widespread group of species with a diversity of traditional medicinal uses in the world. The new findings from *Plectranthus barbatus* ethanolic extracts revealed that cytotoxicity lethality based on *Artemia salina* Leach in roots are active with  $LC_{50}=40.07 \mu\text{g/mL}$  followed by twigs and leaves with  $LC_{50}=66.12 \mu\text{g/mL}$  and  $186.33 \mu\text{g/mL}$  respectively. All plant parts contained Saponins, Terpenoids and Glycosides as a major principal group of secondary metabolites. Extract from Leaves are more active in *Anopheles gambiae* with  $LC_{50}=55.65 \mu\text{g/mL}$  followed by twigs and roots with  $LC_{50}=465 \mu\text{g/mL}$  and  $LC_{50}=636 \mu\text{g/mL}$  at 72 hours exposure respectively. Twig extracts showed moderate effect on both *A. gambiae* and *A. aegyptiae* after 48 hours and 72 hours exposure time with 100% (MRC) Mortality Rate Concentration of  $>1000 \mu\text{g/mL}$ .

## Introduction

Mosquitoes cause more human suffering than any other organism, they transmit several diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis [1]. Here, malaria parasites are transmitted largely by mosquitoes of the *Anopheles gambiae* complex, which is the most efficient vector system in the world. This complex consists of a number of mosquito species that vary in their ability to transmit falciparum malaria in Africa [2]. *Aedes aegypti* is very important vector, transmitting the arbovirus causing dengue haemorrhagic fever (HDF) and chikungunya in human [3]. At present, no effective vaccine is available for both dengue and malaria; therefore, the only way of reducing the incidence of this disease is by mosquito control, which frequently depends on applications of conventional synthetic insecticides which is not biodegradable [4]. On the other side of nature, the plants powder and plant extracts are sometimes more effective and environmental friendly than the synthetic pesticides and these phytochemicals have major role in mosquito control program [5-7].

Malaria is a major global health disease that affects humans, and it predominates in Africa and other areas with tropical climate [8]. Globally, an estimated 3.3 billion people were at risk of malaria in 2011, worldwide there are 104 countries and territories in which malaria is presently considered endemic [9]. In countries where the disease is endemic, pregnant women and children are most at risk. In epidemic areas, both adults and children are at risk [8]. In Tanzania, over 93% of Tanzania mainland population lives in areas where malaria is transmitted. The level of transmission is high in Lake Zone regions, Coastal regions and Southern lowlands. Malaria is still a leading cause of outpatient, inpatient and hospital deaths with 60-80 thousands estimated death per year mainly in children [10].

All over the world an estimated 2.5 billion people are at risk of dengue in tropical and subtropical areas [11]. This disease infection causes a spectrum of symptoms from mild flu-like to severe life threatening haemorrhage [12]. In recent years, Dengue fever and chikungunya affect most parts of the coastal areas of Tanzania and hence increasing burden to the health sector [13,14].

The genus *Plectranthus* (Lamiaceae) represents a large and widespread group of species with a diversity of traditional medicinal uses. The genus comprises a group of around 300 species, distributed in tropical and subtropical areas of Africa, Asia and Australia [15]. One of the interesting species of this genus is *Plectranthus barbatus* Andr., which is well-known for the treatment of various ailments. A diversity of the traditional medicinal uses of *P. barbatus* in India (Hindu and Ayurvedic), East and Central Africa, China and Brazil have been reported to treat many diseases [16]. The majority of uses are for intestinal disturbances and liver fatigue, respiratory disorders, heart diseases and certain central nervous system disorder [16-20]. Clinical studies of the plant and the forskolin constituent support these traditional uses and they have other benefit such as prevention of cancer metastases [21] and for the veterinary purposes [22]. Furthermore, its essential oils in tube has very attractive and delicate odour with spicy which act as an antimicrobial agent [23,24].

The aim of this study was to evaluate the efficacy of ethanolic extracts from *P. barbatus* for larvicidal activity and safety using *Artemia salina* Leach as a test organism in cytotoxicity activity. Moreover, the preliminary screenings of phytochemical constituents are evaluated.

## Materials and methods

### Materials

The plant materials parts (roots, leaves and twigs) of *P. barbatus* species were collected from Temeke District, Dar es Salaam, Tanzania

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in February, 2014. The samples were identified and authenticated in the Department of Botany, University of Dar es Salaam. The Brine Shrimps eggs were purchased from Aquaculture innovations (Grahamstown 6140, South Africa) and sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast. Mosquito larva *Anopheles gambiae* and *Aedes aegyptiae*, were obtained from Tanzania Pesticides Research Institute, TPRI-Arusha, Tanzania, whereas ethanol (absolute) was bought from Flika Chemie GmbH (Sigma-Aldrich\*, Zwijndrecht, Netherlands) and Dimethyl sulfoxide (DMSO), were purchased from Sigma\*. Potassium hydroxide (KOH), Sodium Hydroxide (NaOH), Acetone, Chloroform, were purchased in the Dar es Salaam, Tanzania. Acids (sulphuric acid, Tartaric acid, Hydrochloric acid) were purchased from Sigma-Aldrich\* Zwijndrecht, Netherlands.

### Extraction process

The roots, leaves and twigs of *P. barbatus* were separately dried, grinded to fine powders and later soaked twice in methanol for 48 hours. After that they were separately filtered and further concentrated using a rotary evaporator. The extracts were kept in refrigerator and later subjected to various chemical tests, bioassays and secondary metabolites screening to detect the presence of different useful natural product groups.

### Cytotoxicity lethality assay

*Artemia salina* Leach larvae were used as indicator animals for preliminary cytotoxicity assay of the extracts [25]. The artificial seawater was prepared by dissolving sea salt (3.8 g) in distilled water to make a concentration of 3.8 g/L and then filtered to remove the undesired particles. The media solution was filled into a tank that has been divided into two unequal compartments by perforated polythene wall. Shrimp eggs were later sprinkled into the covered part of the tank and a lamp was illuminated on the uncovered part in order to attract the hatched auto-tropism shrimps. The mature nauplii were collected in between 36 and 48 hours of hatching [25]. The *P. barbatus* extracts were dissolved in Dimethyl sulfoxide (DMSO) in vials in duplicate at an initial concentration 240 µg/mL and decreasing up to 24 µg/mL. In every vial containing the extract in solution, 10 brine shrimp larvae were added. An additional set of vials containing only a solvent (DMSO) in 5 mL of artificial seawater and 10 shrimp larvae acted as control [25]. The number of survived larvae was established after 24 hours and the  $LC_{50}$  values of the concentrations required to kill 50% of the shrimp larvae, the concentrations to give 100% mortality rates concentration (MRC) and confidence intervals (CI) were obtained using probit analysis [26].

### Testing for larvicidal activity

The larvicidal test was performed according to World Health Organization (WHO) protocol with minor modification [27]. The stock solutions (50 mg/mL) of plant extracts were prepared by first dissolving in DMSO. The stock solutions were diluted with distilled water to make 100 mL each, of 500, 250, 100 and 50 µg/mL solutions of plant extracts. Ten late third instars laboratory reared *Anopheles gambiae* and *Aedes aegyptiae* larvae were separately then introduced in the test solution 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 duplicate under controlled temperature ( $25 \pm 2^\circ\text{C}$ ) 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 calculated for each concentration. Thereafter the mortality rate concentrations (MRC) were derived from the regression equations.

### Phytochemical screening of natural product group

Phytochemical screening of natural product groups are performed according to the following standard protocol as described below;

**Screening for saponins:** About 0.5 g of the plants extract was shaken with water in a test tube. Frothing which persist on warming was taken as a preliminary evidence for the presence of Saponins. Few drops of olive oil was added to 0.5 g of extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of saponin [28].

**Screening for tannin:** Into 10 mL of freshly prepared 10% potassium hydroxide (KOH) in a beaker, 0.5 g of extract was added and shaken to dissolve. A dirty precipitate observed indicate the presence of tannin [28–29].

**Screening for alkaloids:** The filtrate was shaken into 3 beakers: Three gram of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was shared into 3 beakers and tasted for alkaloids as follows: Into the first beaker, Hagar's reagent was added into the second beaker; Mayer's reagent was added and into the third beaker Marquin's reagent was added. Precipitations in any of the 3 tests indicate the presence of alkaloid [28,30].

**Screening for cardiac glycoside; (Keller Killiani test):** Total 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 mL of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ). A brown ring obtained at the interface indicates the presence of a de-oxy sugar characteristic of cardenolides [28–30].

**Terpenoids:** 0.2 g of the each extract was mixed with 2 mL of chloroform and concentrated  $\text{H}_2\text{SO}_4$  (3 mL) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicates positive results for the presence of terpenoids [28–30].

### Result and discussion

The phytochemical screening from Table 1 revealed that saponins are present in both roots and leaves; however the activity differs due to the difference in concentration of the compound responsible for the action. These findings are supported by the recent study on phytochemistry, ethanobotanical uses and pharmacology review on the same plant species [15,16]. On the other side the polar group of natural product that is normally water soluble played a big role on the activities. Since on leaves part revealed less or no activity due to lack of this group. Hence the presence of saponins and glycosides as a water soluble natural product group played a big role on these activities as indicated in the Table 1.

Moreover from Table 2; the larvicidal activity in leaves are more active than the other parts with  $LC_{50}$  = 55.65 µg/mL at 72 hours exposure time in *Anopheles gambiae*, this is followed by twigs and roots at  $LC_{50}$  of 465.67 µg/mL and 636.01 µg/mL respectively. Generally, *Aedes aegyptiae* are more resistance towards *P. barbatus* ethanolic extract than

**Table 1.** Phytochemical screening of Plectanthus barbatus Ethanolic extract

Plant parts	Group of natural products				
	Saponins	Tanins	Cardiac glycosides	Alkaloids	Terpenoids
Twigs	√	X	√	√	√
Leaves	√	X	√	X	√
Root	√	ND	ND	√	√

Key: (√) = Presence, (X) = Absence, ND= not done

**Table 2.** Evaluations of Ethanolic extracts of *Plectranthus barbatus* against Late 3rd instars mosquito larvae

Plants part	Time interval(hours)	Anopheles gambiae			Aedes aegyptiae		
		LC <sub>50</sub> (µg/ml)	95%CI	100% MRC	LC <sub>50</sub> (µg/ml)	95%CI	100% MRC
Leaves	24	>1000	NA	>1000	#	NA	NA
	48	177.69	97.42-306.74	>1000	#	NA	NA
	72	55.65	6.49-477.05	591.23	#	NA	NA
Roots	24	#	NA	NA	#	NA	NA
	48	#	NA	NA	#	NA	NA
	72	636.01	486.83-986.02	>1000	>1000	NA	>1000
Twigs	24	>1000	NA	>1000	#	NA	NA
	48	386.66	56.07-6028.02	>1000	>1000	NA	>1000
	72	465.67	67.89-869.56	>1000	>1000	NA	>1000

Key; # = Not active up to maximum concentration of 80 µg/ml, NA= not applicable, MRC = Mortality rate Concentration, LC<sub>50</sub> = Lethal Concentration, CI= Confidence interval

**Table 3.** Cytotoxicity Lethality of *Plectranthus barbatus* extract against *Artemia salina* Leach

CODE NAME	LC <sub>50</sub> (µg/ml)	95% Confidence Interval (µg/ml)	Regression Equation / R <sup>2</sup>	100%MRC (µg/ml)
Leaves	186.33	57.357 – 605.3116	Y=62.30logx-91.44 R <sup>2</sup> =0.983	1182
Twigs	66.12	48.248 – 90.6108	Y=82.84logx-100.8 R <sup>2</sup> =0.903	265
Roots	40.07	22.4295 – 71.5842	Y=125.9logx-151.8 R <sup>2</sup> =0.898	100

Key; R<sup>2</sup> = Retention factor, MRC=Mortality rate concentration, LC= Lethal concentration

*A. gambiae*. The findings revealed that, there is no activity towards *A.egyptiae* up to maximum tested concentration of 80 µg/ml, thus the activity trend are in the order of leaves followed by twigs and roots. In the other lethality (Table 3), it revealed that roots are more toxic towards *Artemia salina* Leach cells with LC<sub>50</sub> of 40.07 µg/ml followed by twigs and leaves with LC<sub>50</sub> of 66.12 µg/ml and 186.33 µg/ml respectively. The activity trend of *P. barbatus* towards brine cells are in order of roots followed by twigs and leaves. These trend comply with the traditional uses of these parts of plant as indicated in the literature [16-20]. Generally, these findings reflect the recently research done in Tanzania in which the plant species showed the potency of mosquito larvicidal in the laboratory trials [31,32].

Thus, the correlation of both mosquito larvicidal and Brine shrimp lethality showed that twigs have mildly activity in both bio-assays while leaves showed more activity on mosquito larvae specifically *A. gambiae*. These findings are more interesting since leaves do not affect the diversity of plants species and consequently is in line with biodiversity conservation. In Brine shrimp test, roots showed remarkable activity correlated to the traditional used.

## Conclusion

These findings revealed that there is no direct connection between cytotoxicity and mosquito larvicidal activity towards the plant parts of *P. barbatus*. Since, leaves showed remarkable mosquito larvicidal activity towards *A. gambiae* and roots possessed strong activity towards *Artemia salina* cells. Thus the plant demonstrate their pharmacological potentials as it has been used for treatments of various diseases since time memorial.

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## Competing interests

The authors declare that they have no competing interests.

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