Tropical Journal of Pharmaceutical Research May 2016; 15 (5): 965-972
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i5.10

Original Research Article

Larvicidal, pupicidal and insecticidal activities of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* mosquitoes

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Received: 22 May 2015 *Revised accepted: 3 April 2016*

Abstract

Purpose: *To evaluate the larvicidal, pupicidal and insecticidal activities of Cosmos bipinnatus, Foenuculum vulgare and Tagetes minuta leaf extracts against Culex quinquefasciatus mosquitoes.*
Methods: *The leaves of the plants were extracted with distilled water, ethanol (95 %), and hexane and the extracts screened for their phytochemical profile. While larvicidal and pupicidal activities were assayed at concentrations ranging from 0.1 - 10 mg/mL, insecticidal property was tested at varying amounts (0.25 - 2 g) of the plant sample. The respective larval mortality was thereafter evaluated using Probit analysis.*
Results: *Saponins, terpenoids, flavonoids and steroids were detected in the plant extracts. The ethanol extracts of F. vulgare, T. minuta and C. bipinnatus exhibited larvicidal activity half-maximal lethal concentration (LC_{50}) of 0.10, 1.17 and 1.18 mg/mL, followed by hexane extracts with LC_{50} value of 1.03, 1.01 and 1.27 mg/mL, respectively, against the larvae of C. quinquefasciatus mosquito. Hexane extracts displayed pupicidal activity with LC_{50} of 1.07, 1.12 and 1.16 mg/mL against F. vulgare, T. minuta and C. bipinnatus, respectively, while the ethanol extracts of T. minuta, C. bipinnatus and F. vulgare displayed pupicidal activity at LC_{50} of 1.11, 1.14 and 1.31 mg/mL respectively, against pupa of C. quinquefasciatus mosquito. The aqueous extracts had no ($p > 0.05$) lethal effects on both larvae and pupa of C. quinquefasciatus at all evaluated concentrations. F. vulgare had the highest ($p < 0.05$) half-maximal knock-down effect (KD_{50} = 7.52 min^{-1}), followed by T. minuta (KD_{50} = 8.64 min^{-1}) on adult C. quinquefasciatus mosquitoes after 6 h of exposure. F. vulgare and T. minuta killed all evaluated mosquito adults within 12 h with LD_{99} = 0.25 g/air, while the leaves of C. bipinnatus had no ($p > 0.05$) knock-down or lethal effects on the adult mosquito.*
Conclusion: *C. bipinnatus, F. vulgare and T. minuta possess larvicidal and pupicidal properties against C. quinquefasciatus, whereas only F. vulgare and T. minuta displayed insecticidal properties. Consequent upon these findings, all the plants can be considered naturally potent larvicidal and pupicidal agents against C. quinquefasciatus.*

Keywords: *Cosmos bipinnatus, Culex quinquefasciatus, Botanical insecticides, Knock-down effect, Larvicidal, Pupicidal, Insecticidal, Foeniculum vulgare, Tagetes minuta*

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INTRODUCTION

Mosquitoes (Diptera: Culicidae) are widely known for their role as vectors of disease-causing pathogens in the world [1]. *Culex quinquefasciatus* are involved in transmitting viral, bacterial and protozoan diseases around the world [2]. The adults of *C. quinquefasciatus* prefer to inhabit areas where there is human dense settlement, larvae dwells in polluted standing water [2]. A study by Berticat *et al* [3] reported that mosquitoes of *C. quinquefasciatus* have developed multiple resistance towards carbosulfan and permethrin insecticides, and that has being caused by resistant gene mutation, wrongful application methods and longer use period of same insecticide. The conventional insecticides have also been reported to be of stable chemical structure with associated deleterious effects on humans and other non-targeted animals [4,5]. Specifically, the resistance shown by *Culex* mosquito larva and adults to insecticides have prompted researches on botanicals to discover new human and eco-friendly insecticides [5]. In addition to being easily biodegradable, non-toxic to other organisms, safer to use, easily available and cost effective [4], the botanical insecticides have been used as alternative methods to control vectors of biological importance [5].

Asteraceae family has a global distribution with 246 genera that are represented by 2300 species in South Africa, and 17 tribes are used for medicinal purposes [6]. The Asteraceae are commercial and traditionally known to produce pesticides, essential oils, ailment medicine, edible food, and some species are used as ornamentals. *Cosmos bipinnatus* Cav. is a half hardy annual herb, with finely cut and thread-like leaves, the solitary inflorescence with pink, purple, red or white flowers [7]. The traditional medicinal uses of *C. bipinnatus* Cav. flower include treatment of various diseases like jaundice, intermittent fever and splenomegaly, and while its triterpene alcohols such as helianol were reported to display anti-inflammatory activity [7]. The Basotho tribe in South Africa traditional make use of *C. bipinnatus* to manage headaches and stomach disorders, while the Afrikaans ethnic group use *C. bipinnatus* to control bed bugs and head lice [8]. On the other hand, the larvicidal and insecticidal properties of *C. bipinnatus* have not being scientifically reported.

The annual hard-woody *Tagetes minuta* L. shrub is used traditionally as a biological pesticide to control agricultural, medicinal and veterinary important insect pests [9]. Larvicidal and insecticidal effects of essential oils from various parts of *T. minuta* against *Aedes aegypti*, *Anopheles stephensi*, *Hyalomma rufipes* (tick) and *Pediculus humanus capitis* (head louse) have been documented [9,10]. Extracts from its floral and foliar parts have also been demonstrated to potentiate good insecticidal activities toward *Phlebotomus duboscqi* (sandfly) [11].

Apiaceae is represented by 78 genera with 368 species in the sub-Saharan region, and genus *Foeniculum* is found worldwide and adds to the 19 endemic Apiaceae genera in southern Africa [12]. The essential oils from *F. vulgare* seeds showed larvicidal activities against mosquitoes of *Anopheles stephensi* and *Culex pipiens* [13].

The present study evaluated leaf formulations of *C. bipinnatus, F. vulgare* and *T. minuta* for their secondary metabolite constituents, larvicidal, pupicidal and insecticidal activities against *Culex quinquefasciatus* mosquitoes.

EXPERIMENTAL

Plant collection

Plants were collected from their plantation in March 2013 around Qwaqwa Township, eastern Free State Province, South Africa. They were identified and authenticated by Dr. AOT Ashafa of the Department of Plant Sciences, University of the Free State, Qwaqwa campus, and voucher specimens ModMed.2013/3, ModMed.2013/4, and ModMed.2013/5 for *Cosmos bipinnatus*, *Foeniculum vulgare*, and *Tagetes minuta*, respectively, were thereafter deposited in the University's Herbarium for future reference.

The fresh leaves of each plant were dried to constant weight in Ecotherm oven (Laboratory Consumables Pty, RSA) at 40 °C prior to pulverizing into fine powder using electric blender (Nanning Mainline Food Machinery Company Ltd, China).

Extract preparation

The powdered samples of each material were divided into three portions of 5 g each and extracted with 150 mL each of distilled water, ethanol (95 %) and hexane, and kept on orbital Labcon Platform shaker (Laboratory consumables, PTY, Durban, South Africa) in lidded 500 mL flasks at 110 rpm for 48 h. The resulting infusion in each case was filtered and evaporated to dryness in a rotary evaporator (Cole-Parmer, Laboratory Consumables and Chemical Supplies Co. Ltd, China) in respect of the organic solvent extracts. The aqueous extracts on the other hand were concentrated over water bath (45 °C). The crude extracts obtained in each case were kept refrigerated (4 °C) prior to use.

Secondary metabolites screening

Adopting standard procedures [14-16], eight secondary metabolites were screened for.

Mosquito collection

Mosquito larvae were collected from Kroonstad in Free State of South Africa from standing water pools, at sites (27°38'27.30" S, 27°11'20.40" E and 1372 m latitude) and (27°39'36.30" S, 27°10'18.90" E and 1338 m latitude). Larvae and female mosquitoes were identified using identification keys as previously described [17]. Museum voucher specimens were prepared for fourth instar larvae (UFSCulex01/Mod2013) and adult female (UFSCulex02/Mod2013) type specimens and deposited at the Department of Zoology and Entomology Museum, University of the Free State, Qwaqwa campus. Mosquito fourth instar larvae were used for larvicidal bioassay evaluation according to WHO [18]. The other earlier larval developmental stages were allowed to develop into adults, while held at temperature of 27 ± 2 °C, humidity of 70 ±1% and a photoperiod regime of 14:10 h (light/dark). To maintain the larval colonies, the aquatic larvae were reared in dechlorinated water and yeast solution.

Larvicidal bioassays

Different extract concentrations (0.1, 0.5, 1, 2, 5, 10 mg/mL) were prepared into separate opened McCartney (20 mL) glass vial for each plant extract. While ten fourth instar larvae were separately pipetted into each graded concentration and served as experimental treatments, the control experiments were conducted using 5 mL of double distilled water without plant extracts. Experimental treatments and control were kept under same conditions that were used to maintain *Culex* mosquito larval colonies as mentioned above. The larval mortalities were counted and recorded after 24 h for analysis. Mosquito larvae were declared dead when it lay flat at the bottom of the vial without motion, or did not respond to any stimuli.

Pupicidal bioassay

Ten two-day old pupa were placed separately into each graded concentration (0.1, 0.5, 1, 2, 5, 10 mg/mL) of each plant extract and represented test treatments. Control experiments were conducted in similar way using 5 mL of double-distilled water. Experimental treatments and control were also kept under same conditions as mentioned above. Pupa mortalities were counted and recorded after 24 h for analysis. Mosquito pupas were declared dead when just floated above water surface without motion, or does not respond to any stimuli.

Insecticidal bioassay

Varying amounts (0.25, 0.5, 1, 1.5, 2 g) of dried powdered leaf material were used. A Whatman No-1 filter paper was cut into a circular (25 mm diameter), and placed under plastic vials cap (25 mm diameter) to fit. The graded concentrations of the powdered leaf were poured onto the filter paper and covered with perforated vial (65.66 mm diameter, area of 13546.55 mm^2) in an inverted position. Ten two day old adult *Culex* mosquitoes were incubated inside the perforated plastic vial. The steps were repeated three times for every tested plant containing the dried plant material and the best two results were recorded at 6 and 24 h and used for analysis.

Data analysis

Probit analysis was used to determine the median lethal concentration (LC_{50}), median lethal dose (LD50), and mean knock-down (KD_{50}). Repetitive-measure of analysis of variance (ANOVA) test was used to determine statistical difference between concentration mortality counts.

RESULTS

Crude extract yield and secondary metabolite constituents

The percentage yield for crude extract of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* were higher in aqueous extracts with 27.00, 23.10 and 13.60 %, followed by ethanolic extracts with 7.00, 11.00 and 7.40 % while hexane extracts had 1.20, 6.0 and 2.40 %, respectively (Table 1).

Saponins, flavonoids, steroids and terpenoids were common for *C. bipinnatus* and *T. minuta* extracts. Tannins were present in aqueous and ethanolic extracts of *F. vulgare*, and alkaloids were detected mostly in *T. minuta* and *F. vulgare* (Table 1).

Larvicidal activity

A similar pattern of complete larvicidal activity was observed at both 5 and 10 mg/µL concentrations of all the investigated extracts.

Table 1: Phytochemical profile of leaf extracts of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta*

Plant Species	Solvents	% Yield	Alk.	Tan.	Phl.	Sap.	Fla.	Ste.	Ter.	Car.
Cosmos bipinnatus	Aqueous	27.00	-	+	-	+	+	+	+	-
	Ethanol	7.00	-	-	-	+	+	+	+	-
	Hexane	1.20	+	-	-	+	-	-	+	-
Foeniculum vulgare	Aqueous	23.10	-	+	-	+	+	+	+	-
	Ethanol	11.00	+	+	-	+	+	-	-	-
	Hexane	6.00	+	+	-	+	-	-	-	-
Tagetes minuta	Aqueous	13.60	+	-	-	+	+	-	+	-
	Ethanol	7.40	+	-	+	+	+	+	+	-
	Hexane	2.40	+	-	-	+	-	-	+	-

Key: + = presence, - = absence, Alk = Alkaloids, Tan = Tannins, Phl = Phlobatannins, Sap = Saponins, Fla = Flavonoids, Ste = Steroids, Ter = Terpenoids, Car = Cardiac glycosides

Generally, the ethanol and hexane extracts exhibited larvicidal activity for *F. vulgare* with LC_{50} values of 0.10 and 1.03 mg/mL, *T. minuta* with LC_{50} values of 1.17 and 1.01 mg/mL, while *C. bipinnatus* had the least larvicidal activity with LC_{50} values of 1.18 and 1.27 mg/mL respectively, against *Culex quinquefasciatus* mosquito larvae after 24 h of exposure (Table 2). The aqueous extracts of all plants had no fatal effects on the larvae at all test concentrations. For the hexane extracts, statistically significant differences existed between larval percentage mortalities at $F_{5,2}$ = 6.46 (p < 0.05) and also between concentration dependent mortalities at $F_{5,2}$ = 5.77 (p < 0.05) (Table 2). There was significant difference for ethanol extracts between plant concentration dependent mortalities at $F_{5,2}$ = 3.57 (p < 0.05), and no significant difference between larval percentage mortalities. A positive correlation was displayed between extract concentrations and larval mortality of larvae for the tested ethanol and hexane extracts (Fig 1A and B).

Table 2: Median lethal concentration indicating larvicidal activities of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* ethanol and hexane extracts against *Culex quinquefasciatus* larvae after 24 h of exposure

		Ethanol extract (% mortality)					Hexane extract (% mortality)				
Conc [mg/µl]	**Int no.**	***C. Bipinnatus***	***F. vulgare***	***T. Minuta***	$F_{5,2}$	***p value***	***C. bipinnatus***	***F. vulgare***	***T. minuta***	$F_{5,2}$	***p value***
0.10	10.00	0.00	100.00	0.	3.57*	0.04	0.00	50.00	20.00	5.77*	0.01
0.50	10.00	40.00	100.00	80	1.86	0.21	10.00	80.00	100.00	6.46*	0.02
1.00	10.00	100.00	100.00	100			10.00	90.00	60.00		
2.00	10.00	100.00	100.00	100			50.00	100.00	100.00		
5.00	10.00	100.00	100.00	100			100.00	100.00	100.00		
10.00	10.00	100.00	100.00	100			100.00	100.00	100.00		
LC_{50}		**1.18**	**0.10**	**1.17**			**1.27**	**1.03**	**1.01**		

Conc = Concentration, Int no. = Initial number, LC_{50} = median lethal concentration, * = significant difference

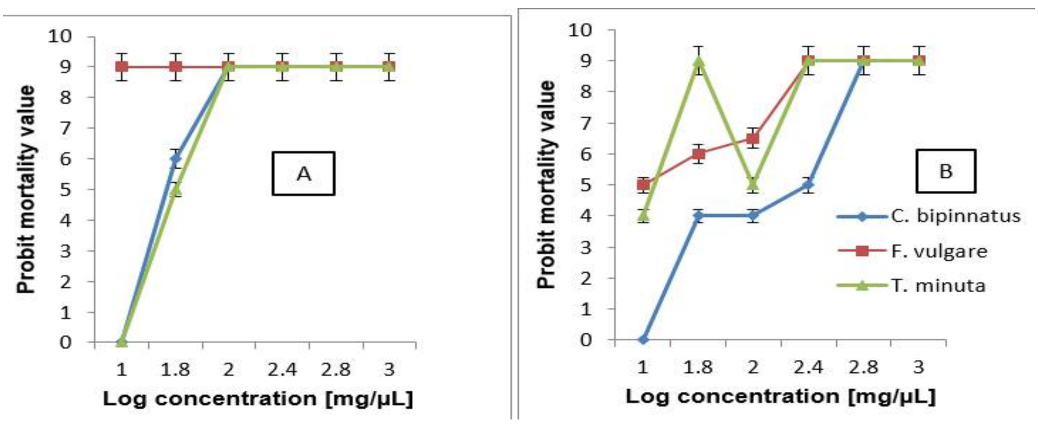

Fig 1: Larvicidal activities of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta*, ethanol (A) and hexane (B) extracts against *Culex quinquefasciatus* larvae after 24 h of exposure

Pupicidal activity

Except for hexane extract of *C. bipinnatus* and *T. minuta*, all other extracts of the two plants (at 5 and 10 mg/µL) elicited uniform and complete 100 % larvicidal activity against the mosquito pupa. Overall, the ethanol and hexane extracts displayed pupicidal activity for *T. minuta* with LC_{50} value of 1.11 and 1.12 mg/mL, *C. bipinnatus* extracts had LC_{50} value of 1.14 and 1.16 mg/mL, and *F. vulgare* with LC_{50} value of 1.31 and 1.07 mg/mL, against *Culex quinquefasciatus* mosquito pupa after 24 h of exposure (Table 3). The aqueous extracts had no fatal effects towards the pupa at all tested concentrations. While the ethanol extracts had significant difference only between plant mortality at $F_{5,2} = 4.29$ ($p < 0.05$), the hexane extracts had significant difference only between pupa percentage mortalities at $F_{5,2} = 3.73$ ($p < 0.05$) (Table 3). Ethanol and hexane extract concentrations displayed positive correlation with percentage pupa mortalities (Fig 2A and B).

Insecticidal activity

Although, *T. minuta* exhibited a dose-dependent knock-down rate with total and most prominent effects at 1.5 and 2 g/air concentration, complete knock-down effects were evidently displayed by *F. vulgare* at concentration range of 1-2 g/air (Table 4). After 6 h of exposure, *F. vulgare* leaves performed the most with an average rate of knock-down effect with KD_{50} = 7.52 min at a knock-down range between 65 - 77 %, while *T. minuta* displayed at KD_{50} = 8.64 min with a knock-down range of 43-55 %, towards adults of *C. quinquefasciatus* mosquitoes. Knock-down rate had significant difference between plant leaves with $F_{4,2} = 10.09$ ($p < 0.01$), while there was no significant difference between plant dose concentration. Also, it is noteworthy that leaves of *F. vulgare* and *T. minuta* killed all evaluated mosquito adults within 12 h of exposure with LD_{99} = 0.25 g/air against *C. quinquefasciatus* mosquitoes adults. However, *C. bipinnatus* leaves had no knock-down or fatal effects on mosquito adults.

Table 3: Pupicidal activities with median lethal concentrations of ethanol and hexane extracts of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* larvae after 24 h of exposure

		Ethanol (% mortality)					Hexane (% mortality)				
Conc (mg/µl)	Int no.	*C. bipinnatus*	*F. vulgare*	*T. minuta*	$F_{5,2}$	*p value*	*C. bipinnatus*	*F. vulgare*	*T. minuta*	$F_{5,2}$	*p value*
0.10	10.00	20.00	70.00	50.00	3.14	0.06	50.00	40.00	60.00	3.73*	0.04
0.50	10.00	20.00	100.00	100.00	4.29*	0.05	10.00	100.00	50.00	2.06	0.18
1.00	10.00	30.00	100.00	90.00			60.00	80.00	90.00		
2.00	10.00	100.00	100.00	100.00			90.00	100.00	100.00		
5.00	10.00	100.00	100.00	100.00			80.00	100.00	90.00		
10.00	10.00	100.00	100.00	100.00			100.00	100.00	90.00		
LC_{50}		**1.14**	**1.31**	**1.11**			**1.16**	**1.07**	**1.12**		

Conc = Concentration, Int no. = Initial number, LC_{50} = median lethal concentration, * = significant difference

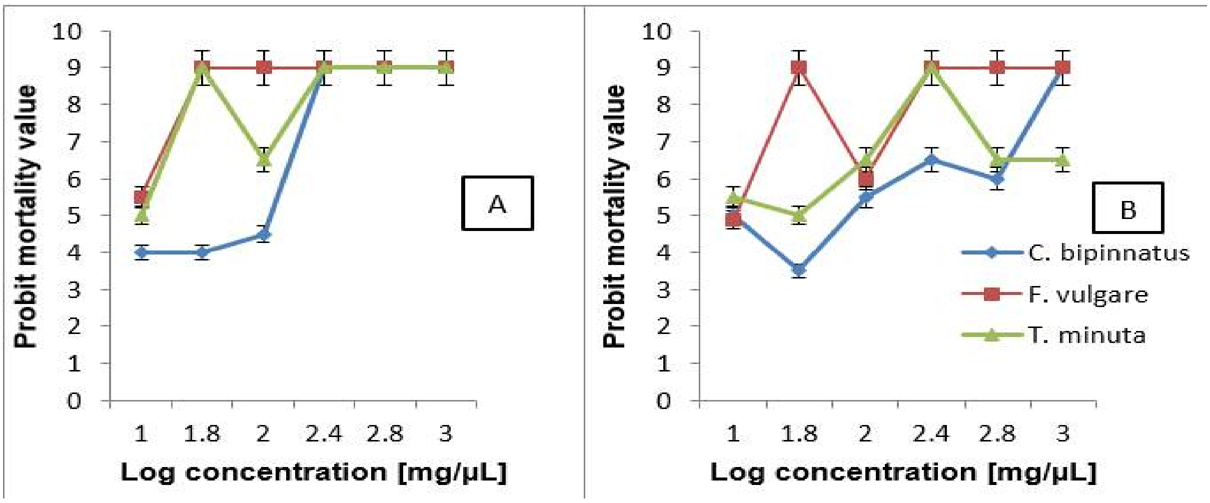

Fig 2: Pupicidal activities of ethanol (A) and hexane (B) extracts of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* mosquito pupa after 24 h of exposure

Table 4: Median lethal dose and knock-down of leaf powder of *Cosmos bipinnatus, Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* adult mosquitoes after 6 and 12 h exposure periods

		Knock-down (%)				
Dose (g/air)	**Int no.**	***Cosmos bipinnatus***	***Foeniculum vulgare***	***Tagetes minuta***	$F_{4,2}$	***P-value***
0.25	10.00	0.00	0.00	0.00	3.16	0.08
0.50	10.00	0.00	70.00	30.00	10.09*	0.01
1.00	10.00	0.00	100.00	50.00		
1.50	10.00	0.00	100.00	100.00		
2.00	10.00	0.00	100.00	100.00		
LD_{50}		-	***7.52***	***8.64***		

Int no. = Initial number, LD_{50} = median lethal dose, * = significant difference

DISCUSSION

The aquatic immature larvae stage is recognised as the most vulnerable and best control strategy to effectively reduce mosquito population densities during infestations [2]. Ethanol and hexane leaf extracts of *F. vulgare, T. minuta* and *C. bipinnatus* displayed good larvicidal and pupicidal activities, and may be considered effective for larvae control since their LC_{50} values were lower than 100 mg/mL [19]. *C. bipinnatus* and *T. minuta* ethanol leaf extracts contained terpenoids, flavonoids, saponins, steroids and tannins that play a role in plant defence against insect pests, and might have been responsible for larval and pupa deaths. Ethanol extracts of *Citrus sinensis* have displayed both larvicidal and pupicidal activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes [20], and similar bioactivities were observed for *F. vulgare, C. bipinnatus* and *Tagetes minuta* ethanol extracts in this study against larva and pupa of *C. quinquefasciatus* mosquitoes. *F. vulgare* seed essential oil extract was reported for larvicidal activity on both 2nd and 4th larval instar of *Culex pipiens* [21], and the non-polar compounds present in the hexane extract can similarly act on larvae of *C. quinquefasciatus* mosquitoes.

F. vulgare, *C. bipinnatus* and *T. minuta* aqueous leaf extracts had no fatal effects against *C. quiquefasciatus* larvae and pupa. Similar trends were observed in previous larvicidal activity studies, where ethanol and hexane leaf extracts of *Cassia occidentalis* and *Lantana camara* displayed better larvicidal activities toward *Anopheles stephensi* and *Aedes aegypti* larvae, respectively than aqueous extracts [21,22]. The aquatic immature life stages (larvae and pupa) prefer to inhabit water pools that are polluted [23], and such behaviour may act to assist larvae and pupa to develop metabolic defence strategies against plant aqueous extracts that they might have been exposed to previously. This observation may be attributed to the differences in polarity of the extractants used in this study [7,22].

Generally, the attributes elicited by all the investigated plants in this study may be adduced to their secondary metabolites as revealed by the results of the phytochemical analyses. The biologically active terpenoids of *T. minuta* extracts such as dihydrotagetone, tagetones, ocimenones and piperitone have been reported for possible synergistic larvicidal effects [9]. The phytonutrients of *F. vulgare* foliage extracts were also found to be toxic and effective against *Anopheles dirus* and *Aedes aegypti* mosquitoes [24]. These phytonutrients have been opined to penetrate mosquito integument to disrupt important metabolic reactions. Such disruption may deprive adult mosquitoes of oxygen and death resulting from suffocation could occur [25]. This was observed at higher investigated concentrations of *T. minuta* and *F. vulgare* in this study. Extracts or chemicals with respective KD_{50} and LD_{50} values of lower than 20 min and 80 g/air have been pharmacologically adjudged to be potent insecticides [5]. Therefore, *T. minuta* and *F. vulgare* leaves powder could be said to have exhibited rapid and effective insecticidal activity towards *C. quinquefasciatus* adult mosquitoes.

CONCLUSION

F. vulgare, C. bipinnatus and *T. minuta* leaf extracts exhibit larvicidal and pupicidal activities against aquatic *C. quinquefasciatus* larvae and pupa, but the aqueous extracts do not. Only *F. vulgare* and *T. minuta* dried extracts has insecticidal activity against adult *C. quinquefasciatus* mosquitoes. Further studies should be conducted to monitor and evaluate the mode of action at the molecular level for each plant extract to clarify the observed effects in this study. Since *F. vulgare, C. bipinnatus* and *T. minuta* plants as well as *C. quinquefasciatus* mosquitoes are widely distributed around the world, these plants have a potential to be used in local communities for mosquito control during breeding and infestation seasons, thus promoting the use of natural pesticides.

ACKNOWLEDGEMENT

The authors acknowledge National Research Foundation (NRF) Scarce Skills, and Prof. NJL Heideman, Dean, Faculty of Natural and Agricultural Sciences, for bursary, as well as UFS-Qwaqwa Research Committee which provided funding (grant no. 211427604).

CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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