

# Larvicidal efficacy of some *Tephrosia* species extracts against *Anopheles Gambiae* and *Culex Quinquefasciatus* Say

## *Anopheles Gambiae* ve *Culex Quinquefasciatus* Say türü sivrisinek larvalarına karşı *Tephrosia* türü bitki ekstraktlarının etkinliği

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### ABSTRACT

**Aim:** The study was conducted to evaluate the effect of some *Tephrosia* species against the larvae; *Anopheles gambiae* and *Culex quinquefasciatus*. These plants have proven to have insecticidal properties and regenerate quickly. Reduction of mosquito borne diseases by killing mosquitoes at the larva stage is bio-control alternative method in the rural area since most of people cannot afford mosquito treated nets.

**Methods:** The mosquito larvicidal test was performed on the two species by using ethanolic phyto-extracts according to World Health Organization (WHO) protocol with minor modifications.

**Results:** Activity study of *Tephrosia vogelii* has shown that leaves are more active than other parts of the plants. The *anopheles* species exhibited more resistance compared to *culex* species by the factor of 4. The crude extract from the leaves of *Tephrosia vogelii* revealed 100% mortality rate against *Anopheles gambiae* at 235µg/ml and LC50 of 94.77µg/ml compared to 100% mortality rate against *Culex quinquefasciatus* at 55µg/ml and LC50 of 19.696µg/ml. The inter-species activity comparison indicated that larvicidal potential of *Tephrosia* species evaluated are in order of *T. vogelii* > *T. villosa* > *T. aequilata* > *T. pumila*.

**Conclusion:** The findings revealed that, these species are potential alternative bio-control agents in the control of the mosquito borne diseases. Leaves exhibited better activity than other parts of the plant and the trends of the species activity towards both mosquito larvae after 72h exposure time are as followed: *T. vogelii* > *T. villosa* > *T. aequilata* > *T. pumila*. Generally, *Anopheles gambiae* larvae were more resistant than *culex quinquefasciatus*. It is interesting to mention that, our results are comparable to the recently discovered larvicidal product called Bacillus Thuringiensis, subsp. Israeliensis (BTI®) claimed to be specific to targeted mosquito larva.

**Keywords:** Larvicidal; *Anopheles gambiae*; *Culex quinquefasciatus*; *Tephrosia villosa*; *Tephrosia pumila*; *Tephrosia vogelii*; *Tephrosia aequilata*.

### ÖZET

**Amaç:** Çalışmanın amacı, *Anopheles gambiae* ve *Culex quinquefasciatus* türü sivrisinek larvalarına karşı *Tephrosia* türü bitki ekstraktlarının etkinliğinin araştırılmasıdır. *Tephrosia* bitki türlerinin insektisit özellikleri bulunmakta ve iyileştirmeyi hızlandırmaktadır. Kırsal bölgelerde, larva aşamasındaki sivrisineklerin öldürülmesi, sivrisineklerden kaynaklanan hastalıkların kontrolünde alternatif bir yöntemdir.

**Yöntemler:** Küçük değişiklikler yapılmış Dünya Sağlık Örgütü (WHO) protokolüne göre, etanolik bitki ekstraktı kullanılarak sivrisineklere larvisidal test yapıldı.

**Bulgular:** *Tephrosia vogelii* bitkisinin yaprakları diğer bitki bölümlerine göre daha fazla aktivite göstermekte idi. Faktör 4'ten dolayı *anopheles* türü sivrisineklere *culex* türüne göre daha fazla direnç ortaya koydu. *Tephrosia vogelii* yapraklarından elde edilen ekstrakt 235µg/ml dozunda *Anopheles gambiae* türü üzerinde mortalite göstermekte ve LC50, 94.77µg/ml idi. Buna karşılık, 55µg/ml konsantrasyonda *Culex quinquefasciatus* üzerinde mortalite göstermekte ve LC50, 19.696µg/ml idi. *Tephrosia* türlerinin larvisidal etkileri sırasıyla *T. vogelii* > *T. Vilosa* > *T. Aequilata* > *T. Pumila* şeklinde idi.

**Sonuçlar:** Sonuçlar, çalışılan bitki türlerinin sivrisinek kaynaklı hastalıkların kontrolünün sağlanmasında alternatif olabileceğini göstermektedir. Yapraklar diğer bitki bölümlerinden daha fazla aktivite göstermekte idi ve her iki sivrisinek türü larvalarının 72 saat ekstrakta maruz kalması sonucunda elde edilen bitki ekstraktlarının gücü sırasıyla *T. vogelii* > *T. Vilosa* > *T. Aequilata* > *T. Pumila* idi. *Anopheles gambiae* türü sivrisinek larvaları *culex quinquefasciatus*'a göre daha dirençli idi. Sonuçlarımız, son zamanlarda ortaya çıkan ve sivrisinek larvalarını hedefleyen Bacillus Thuringiensis, subsp. Israeliensis (BTI®) ile karşılaştırılabilir.

**Anahtar kelimeler:** Larvisidal; *Anopheles gambiae*; *Culex quinquefasciatus*; *Tephrosia villosa*; *Tephrosia pumila*; *Tephrosia vogelii*; *Tephrosia aequilata*.

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## INTRODUCTION

Malaria and other vector-borne diseases like filariasis contribute to the major disease burden in Tanzania and the World [1,2]. Mosquitoes transmit serious human diseases like malaria, filariasis, Japanese encephalitis, dengue haemorrhagic fever and yellow fever that cause millions of deaths every year [1]. According to the national malaria control program approximately 17 to 20 million cases occur in Tanzania per year, accounting for over 40% of all outpatient attendance. From those cases, more than 80,000 people die from the disease each year, thus it is a major killer of children under five year's old [1]. Globally, there are at least 300 million acute cases of malaria each year resulting in about one million deaths [3].

Biological control at the larva stage of development of mosquitoes is one of the techniques which afford a cheap, easy to use and environment friendly method of malaria control and other mosquito borne diseases [4]. Numerous plant products have been reported either as insecticides for killing larvae, adult mosquitoes or as repellents for mosquito biting and are one of the best alternatives for mosquito control [3]. For instance *Lepidagathis alopecuroides* was shown to possess activities on both *Anopheles gambiae* and *Culex quinquefasciatus* while *Azadirachta indica* showed potent activities only on *Culex quinquefasciatus* after a long exposure until the emergence of the adult, and these indicate that the *L. alopecuroides* is more potent than neem [4]. During the last decade, various studies on natural plant products against mosquito vectors have indicated them as possible alternatives to synthetic chemical insecticides [5,6-16]. Natural products from plants origin with insecticidal properties have been used in recent years for control of variety of pest insects and vectors, for example the findings from the *Momordica charantia* revealed the mosquito larvicidal properties against *Culex quinquefasciatus* Say [17], and the recently findings conducted in Tanzania on *Harrisonia abyssinica* against *Culex quinquefasciatus* Say revealed the promising results [18]. However, more concerted efforts have to go into these studies to make these environment-friendly natural products viable for field use and for large-scale vector control operations.

The genus *Tephrosia* consists of flowering plants in the family Papilionaceae. Many species in the genus are poisonous, particularly to fish due to their concentration level of rotenone. These species historically have been used by indigenous culture as fish and insecticidal toxins [19-22]. In the last century several species have been studied in connection with the use of

Rotenone as an insecticide and pesticide [23-26]. However, to the best of our knowledge there is little research on the mosquito larvicidal efficacy on the crude

or compounds isolated from the genus. Here we report the larvicidal activity of organic extracts of four *Tephrosia* species commonly found in Tanzania in comparison with recently discovered larvicidal product Thuringiensis, subsp. Israeliensis (BTI®) [27,28]. The species investigated were *Tephrosia villosa* (L.) Pers., *Tephrosia pumila* (Lam.) Pers., *Tephrosia vogelii* Hook.f. and *Tephrosia aequilata* Baker..

## MATERIALS AND METHODS

### Materials

Ethanol (absolute) was supplied by Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands), Dimethyl sulfoxide (DMSO) was purchased from Sigma® Poole, Dorset, UK. Mosquito larva, *Culex quinquefasciatus* Say., were obtained and reared at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences whereas *Anopheles gambiae* were obtained from the National Institute for Medical Research, Amani Research Center. *Bacillus thuringiensis, subsp. israeliensis* (BTI®) were obtained from the Malaria Research center, Mikocheni Dar es Salaam.

### Collection and preparation of plant Materials

The *Tephrosia* species were collected from different parts of Tanzania, *T. villosa* were collected from Kibaha-Coast region, *T. vogelii* from Muheza-Tanga region, *T. aequilata* from Rudewa-Rukwa region whereas *T. pumila* was collected from Kilosa-Morogoro region. The plant species were identified by Mr. Haji Selemani, a senior botanist from the Department of Botany, University of Dar es Salaam. The plant materials were dried under shade and then pulverized by milling machine to get powders for extraction.

### Extraction Process

The plants powders from selected *Tephrosia* species were extracted by using absolute ethanol for 24 hours repeated two times. The extracts were then dried under *vacuo* using rotary evaporator and stored at -20°C until the time for testing. The standard BTI® was ground into fine powder by using paste and mortar and the stock solutions were prepared by using distilled water.

### Laboratory testing for larvicidal activity

The mosquito larvicidal test was performed according to World Health Organization (WHO) protocol with minor modifications [29,30]. Stock solutions of about 50mg/ml of each plant extract were prepared by dissolving in DMSO. The stock solutions were diluted with distilled water to make 100ml each of 500, 300, 100, 50, 30 up to 3µg/ml solution of each plant

extract including the Standard *Bacillus Thuringiensis*, subsp. *Israeliensis* (BTI®). The late ten 3<sup>rd</sup> or newly emerged 4<sup>th</sup> instar laboratory reared *Culex quinquefasciatus* and *Anopheles gambiae* mosquito larvae were then introduced in the test solution and mortality was observed after 24, 48, 72hours. Negative control tests contained mosquito larvae, DMSO (0.5%) and water only. All tested experiments were carried out in duplicate under controlled temperature (25 ±2 °C) and relative humidity of 75-85%. The larvae were considered dead if they were immobile, unable to reach the water surface and lacked head to tail flexion in response to tapping the beaker with the end of pencil. The larvae were scored for percentage mortality after 24, 48 and 72 hours.

### Data analysis

#### Mosquito Larvicidal analysis

The percentage mean mortality rate (% MR) was determined using the equation:

$$\%MR = \frac{\text{Mean death count}}{\text{Initial larvae population}} \times 100$$

Their concentrations were introduced in the Probit analysis for the evaluations of regression equations, lethal concentrations for the half of test organisms (LC<sub>50</sub>), 95% confidence interval and ultimately the 100% mortality rate concentration (100% MRC) were delivered from the data [31].

### RESULTS

The results obtained from this study are summarized in Tables 1 and 2. Generally laticidal potential of *Tephrosia* species are in order of *T. vogelii* > *T. villosa* > *T. aequilata* > *T. pumila*. Specifically, activity trend of *T. vogelii* showed that leaves are more active than fruits followed by stem. By considering the sensitivity of the tested larvae; *Anopheles* species exhibited more resistance compared to *Culex* species by the factor of 4, elaborated by results obtained from the most active crude extracts of *Tephrosia vogelii* leaves; where 100% mortality against *Anopheles gambiae* was 235µg/ml and LC<sub>50</sub> of 94.77µg/ml compared to 55µg/ml and LC<sub>50</sub> of 19.696µg/ml against *Culex quinquefasciatus*.

**Table 1:** Laboratory evaluations of Ethanolic extract of *Tephrosia* species against late III instars *Anopheles gambiae* larvae.

Tephrosia Species	24 h (Day 1)			48 h (Day 2)			72 h (Day 3)		
	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100% MRC (µg/ml)	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100% MRC (µg/ml)	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100% MRC (µg/ml)
TVLE	94.77	47.57–251.34	235	13.70	9.34 – 21.69	55	5.27	4.78 – 5.83	11
TVSE	132.34	97.01 – 208.06	287	138.23	101.64–219.97	250	21.17	11.79 – 36.24	105
TVFE	>500	NA	>1000	159.69	140.24–188.40	500	67.60	32.41–186.35	181
TASE	#	NA	#	104.83	65.65 – 166.14	181	36.83	26.67 – 52.87	100
TALE	#	NA	#	60.42	39.84 – 92.38	235	21.65	16.45 – 57.90	105
TPtwE	#	NA	#	#	NA	#	138.45	74.66 – 331.14	500
TPLE	#	NA	#	#	NA	#	#	NA	#
TVitwE	>500	NA	>1000	200.98	113.42–786.24	363	44.72	19.92 – 87.15	133
TViLE	>500	NA	>1000	>500	NA	>1000	92.70	41.56 – 376.89	250
TViFE	63.80	57.61 – 69.86	105	44.49	40.77 – 48.41	105	36.48	30.35 – 43.34	105
BTI®	1.06	0.75 – 1.36	10	0.682	0.396 – 0.967	10	<0.682	NA	<10

TVLE=*Tephrosia vogelii* leaves ethanol, TVSE=*Tephrosia vogelii* stem ethanol, TVFE=*Tephrosia vogelii* fruits ethanol, TASE=*Tephrosiaaequilata* stem ethanol, TALE= *Tephrosia aequilata* leaves ethanol, TPtwE= *Tephrosia pumila* twigs ethanol, TPLE= *Tephrosia pumila* leaves ethanol, TVitwE=*Tephrosia villosa* twigs ethanol, TViLE=*Tephrosia villosa* leaves ethanol, TViFE=*Tephrosia villosa* fruits ethanol, BTI®= *Bacillus Thuringiensis*, subsp *Israeliensis*, # = NO mortality observed at maximum tested concentration and time. NA =Not applicable.

**Table 2.** Laboratory evaluations of Ethanolic extract of Tephrosia species against late III instar *Culex quinquefasciatus* larvae.

Tephrosia species	24 h (Day 1)			48 h (Day 2)			72 h (Day 3)		
	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100% MRC (µg/ml)	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100%MRC (µg/ml)	LC <sub>50</sub> (µg/ml)	95% (CI). Limits (Lower-upper)	100% MRC (µg/ml)
TVLE	19.69	10.45 – 33.69	55	17.92	12.59 – 24.71	55	15.20	6.66 – 35.10	52
TVSE	37.88	25.59 – 50.25	90	37.78	21.92 – 62.66	90	35.48	22.15 – 53.06	71
TVFE	24.83	14.63 – 39.89	62	21.01	11.82 – 37.64	55	12.73	7.68 – 20.68	52
TASE	>500	NA	>1000	>500	NA	>1000	>500	NA	>1000
TALE	>500	NA	>1000	>500	NA	>1000	>500	NA	>1000
TPtwE	#	NA	#	#	NA	#	#	NA	#
TPLE	47.43	31.96 – 82.14	142	31.43	15.86 – 80.89	105	20.52	8.03 – 59.63	83
BTI®	0.229	0.172 – 0.293	0.890	0.198	0.168 – 0.235	0.880	0.176	0.103 – 0.261	0.468

TVLE=*Tephrosia vogelii* leaves ethanol, TVSE=*Tephrosia vogelii* stem ethanol, TVFE=*Tephrosia vogelii* fruits ethanol, TASE=*Tephrosiaaequilata* stem ethanol, TALE= *Tephrosia aequilata* leaves ethanol, TPTwE= *Tephrosia pumila* twigs ethanol, TPLE= *Tephrosia pumila* leaves ethanol, BTI®= *Bacillus Thuringiensis, subsp Israeliensis*.# = NO mortality observed at maximum tested concentration and time. NA=Not-applicable,LC<sub>50</sub>=Lethal concentration, CI= Confidence interval, MRC= Mortality rate concentration

## DISCUSSION

The literature review shows that *T. vogelii* is considered an insecticide and a pesticide due to the presence of Rotenone as a major compound and the activity may be caused by the presence of this compound in varying concentrations in these species [32]. The results are similar to that reported in the *Neurautanenia mitis* (Lidupa) against mosquito larvae in Tanzania [33,20]. On the other hand previous study revealed that fruits of *T.villosa* showed milder activity in *C. quinquefasciatus* say than other plant parts[36], while insecticidal activities of *T.vogelii* reported, match with the observed activities of this plant species[19]. More interesting is the possession of larvicidal activity in the leaves whose harvesting do not affect the diversity of the plant and consequently is in line with biodiversity. Hence the plant stands a better chance to be included in the intervention programmes against mosquito larvae.

The other Tephrosia species (*T. villosa*, *T. aequilata* and *T. pumila*) have been used for the treatment of various diseases such as diabetes, gonorrhoea and ulcer [34,35]. Due to these medicinal uses they may lack an insecticidal and pesticidal activity as proven in the results from the Table 1 and 2. Also the findings have shown that larvicidal activities of the extracts of these species vary according to the plant species and part of the plant used. However, this is the first report on the comparison of larvicidal activities of Tephrosiaspecies in Tanzania.

## CONCLUSION

It is evident that among all four Tephrosia species tested, the leaves of *T. vogelii* possess remarkable larvicidal activity at different time of exposure. Larvicides of great value could be developed from this plant as an alternative means to control mosquitoes and thus help combat malaria and filariasis especially in the rural area where people can't afford to have a mosquito treated net as well as proper modern treatment due to poverty and inadequate medical facilities and medicine. Our team is still working on the safety on non targeted organisms and the environment. When safety is established the pools of water around human habitats can be inoculated with extracts of these plants species as they potent control against the *Brancroftian filariasis* vector of *C.quinquefasciatus*, and more importantly, the malaria *A. gambiae*.

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## REFERENCES

1. [www.malariafreefuture.org/projects/Tanzania/index.php](http://www.malariafreefuture.org/projects/Tanzania/index.php). Accessed on October, 2011.
2. World Health Organization: World Malaria Report. Geneva 2008.
3. RBM; [www.rollbackmalaria.org](http://www.rollbackmalaria.org). Accessed on October, 2011.
4. Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI. Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus* (Say). *Afr J Biotechnol.* 2006; 5(9): 761-65.
5. Davidson G. Alternative measures to insecticides for mosquito control. *Pesticide Sci.* 1972; 3: 503-14.
6. Palsson K, Janeson TGT. Plant products used as mosquito repellents in Guinea Bissu, West Africa. *Acta Tropica.* 1999; 72: 39-52.
7. Deshmukh PB, Chavan SR, Renapurkar DM. A study of insecticidal activity of twenty indigenous plants. *Pesticides.* 1982; 7-10.
8. Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vectors. *ICMR Bull.* 2003; 33(1): 1-10.
9. Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula*. *Bioresource Technol.* 2005; 37: 1235-40.
10. Singh RK, Mittal PK, Dhiman RC. Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family: Asclepiadaceae) against mosquito larvae. *J Com Dis.* 2005; 37:109-13.
11. Thomas TG, Raghavendra K, Lal S, Saxena VK. Larvicidal properties of latex from unripe fruits of *Carica papaya* Linn (Caricaceae). *J Com Dis.* 2004; 36(4): 290-2.
12. Singh SP, Raghavendra K, Singh RK, Subbarao SK. Studies on the larvicidal properties of leaf extract of *Solanum nigrum* Linn (Family: Solanaceae). *Curr Sci.* 2001; 81(12): 1529-30.
13. Mehra BK, Haridhar PK. The effect of crude acetone extract of *Annona squamosa* Linn (Family: Annonaceae) on possible control potential against larvae of *Culex quinquefasciatus* Say. *J Entomol Res.* 2001; 24(4):141-6.
14. Thomas TG, Sharma SK, Jalees S, Rehman SJ. Larvicidal properties of an indigenous plant *Yucca aloifolia* Linn against mosquito larvae. *J Basic Appl Bio Med.* 1994; 2:53-5.
15. Pathak N, Mittal PK, Singh OP, Sagar V, Vasudevan P. Larvicidal action of essential oil from plants against the vector mosquito, *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L). *Intl Pest Contr.* 2000; 46:53-5.
16. Maniafu BM, Wilber L, Ndiege IO, Wanjala CC, Akenga TA. Larvicidal activity of extracts from three *Plumbago* spp against *Anopheles gambiae*. *Mem Inst Oswald Cruz, Rio de Janeiro.* 2009; 104(6): 813-7.
17. Singh RK, Dhiman RC, Mittal PK. Mosquito larvicidal properties of *Momordica charantia* Linn (Family : Cucurbitaceae). *J Vect Borne Dis.* 2006;43: 88-91.
18. Innocent E, Magadula JJ. Variations of anti-Mosquito larvicidal constituents in the *Harrisonia abyssinica* species of Tanzania. *Int J Biol Chem Sci.* 2010; 4(1): 218-25.
19. U.S. Food & Drug Administration (2006-03) Results for search term "tephrosia" (html). FDA POISONOUS Plants Database US. Food & Drug Administration. accessed on October 2010.
20. Puyvelde LV, De Kimpe N, Mudaheeranwa JP, Gasiga A, Schamp N, et al. *Journal of Natural Products.* 1987; 50:349.
21. Mihale MJ, Kidukuli AW, Selemeni HO. Indigenous knowledge in the Management of Pests in Tanzania. The role of traditional knowledge in pest management. Lambert Academic Publishing group. 2010; 14-20.
22. Mihale MJ, Deng AL, Selemeni HO, Mugisha-Kamatanesi M, Kidukuli AW, Ogendo JO. Use of indigenous knowledge in the management of field and storage pests around Lake Victoria basin in Tanzania. *Afr J Environ Sci Technol.* 2009;3(9):251-9.
23. Genus *Tephrosia* Pers. Germplasm Resources Information Network. United State Department of Agriculture. Accessed on October, 2007.
24. Qualtrocchi, Umberto (2000).CRC World Dictionary of Plant Names, Scientific Names, Eponyms, and Entomology. IV R-Z. Taylor & Francis US, p.2642. ISBN 978-0-8493-26783.
25. Bussman RW. Plant use of Maasai of Sekenanivalley, Maasai Mara, Kenya. *J Ethnobiol Ethnomed.* 2006; 222.
26. GRIN. Species Records of *Tephrosia* Gerplasm Resources Information Network. United State Department of Agriculture. Accessed on October, 2010.
27. BTI: *Bacillus Thuringiensis*, subsp. *Israeliensis*; Valent Biosciences Corporation, 870 Technology Way. Libertyville, IL 60048 USA.
28. Geissbuhler Y, Kannady K, Chaki PP, Emidi B, Govella NJ, Myagaya V, et al. Microbial larvicide applications by a large-scale, community-based program reduce malaria infection prevalence in urban Dar es salaam, Tanzania. *PLoS ONE.* 2009; 4(3): e5107.
29. WHO, World Health Organization: Protocol for Laboratory and Field Evaluation of Insecticides and Repellents: Report of the WHO Informal Consultation on the evaluation and testing of Insecticides. Ref: CTD/WHOPES/IC/96.1. Geneva 1996.
30. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC 81: 807.
31. Finney DJ. Probit analysis, III edn. London: Cambridge University Press 1971; 1-333.
32. Touqeer S, Saeed MA, Ajaib M. A Review on the Phytochemistry and Pharmacology of Genus *Tephrosia*. *Phytopharmacology.* 2013; 4(3): 598-637.
33. Mayunga HH Nkunya, Natural Chemicals for Disease and Insect Management; Professorial Inaugural Lecture, University of Dar es salaam, Tanzania: 2002; 130-134.
34. Jain SR, Sharma SN. Hypoglycemic Drugs of India Indigenous Origin. *Planta Medica* 1967; 15(4): 439-42.
35. NAPLALERT DATABASE: University of Illinois, Chicago; 2011.
36. Nondo RSO, Mbwambo ZH, Kidukuli AW, Innocent EM, Mihale MJ, Erasto P, et al. Larvicidal, antimicrobial and brine shrimp activities of extracts from *Cissampelos mucronata* and *Tephrosia villosa* from coast region, Tanzania. *BMC; Complementary and Alternative Medicine:* 2011; 11:33.