Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Chemistry

Elixir Appl. Chem. 52 (2012) 11263-11265

ISSN: 2229-712X

Chemical constituents, toxicity and larvicidal activity of the essential oil from the leaves of acalypha hispida and acalypha wilkesiana in south-west Nigeria

Sherifat Aboaba and Olukemi Omotoso
Department of Chemistry, University of Ibadan, Nigeria.

ARTICLE INFO

Article history:
Received: 27 August 2012;
Received in revised form:
13 October 2012;
Accepted: 30 October 2012;

Keywords
Acalypha hispida,
Acalypha wilkesiana,
Monoterpenes,
Artemia salina,
Anophelis gambiae.

ABSTRACT
The chemical composition of the essential oils from the leaves of *Acalypha hispida* and *Acalypha wilkesiana* obtained by hydro distillation, were analyzed by Gas chromatography linked with Mass spectrometry. The main constituents of the essential oil from *A. hispida* were neral (11.04%), citral (12.87%), 6,10,14, trimethyl-2-pentadecanone (13.43%) and n-hexadecanoic acid (14.69%) while neral (30.66%) and citral (36.10%) which are monoterpenes were the major compounds in the oil of *A. wilkesiana*. The essential oils were tested for toxicity against brine shrimps larvae (*Artemia salina*) and showed LC_{50} values of 122.28µg/mL and 212µg/mL respectively while their activity against *Anophelis gambiae* reveal LC_{50} values of 125µg/mL and 83.33µg/mL respectively.

Introduction

Acalypha wilkesiana is a lax shrub which grows up to 5 m high, native of tropical Asia. It is grown in the region, usually as a variegated-leaf cultivar, and as an ornamental for its red catkins and leaf form. In Trinidad a leaf-poultice is deemed good for headache, swellings and colds [9]. The leaf-extract is active against Gram +ve bacteria and is used in Agri-horticulture [10,11]. The leaf is used for medicines such naso-pharyngeal affections and pain-killers [5,8].

Acalypha hispida (red-hot cat tail), originated in Oceania, but has become naturalized to multiple countries in North America, including the United States, Mexico, and Belize. It can grow 1.8 to 3.7 meters tall, and have a spread of 0.9 to 1.8 meters. The plant has become somewhat domesticated, due to the nature and color of its flowers. It can be grown from seeds as well as from cuttings. It can be kept either as an outdoor plant or as a houseplant [9]. The phytochemical constituents of the leaves suggested diverse biological activities including antimicrobial, anticancer, antitumor effect [5].

There are several reports on the phytochemical analyses, essential oil composition of other parts and biological activities of the two studied plants [13,15,14,5,6,7,4, 17 and 20] but none on the leaf essential oils. Interest in the control of Anopheles gambiae lies in the fact that it acts as a vector of malaria fever, a great menace in Africa and in particular, Nigeria. Larvicides are products that kill mosquito larvae. Targeting larvae is more desirable than controlling adults because the larvae are concentrated in a relatively small area; whatever microbial insecticide adopted needs to be consumed by mosquito larvae and must be applied well before the last larval instar stage. Essential oils from plant have been documented to act as larvicides [19,21,and 22]

As part of a continuous research program on plants from south west Nigeria [1], this paper reports the volatile compounds obtained from the leaves of A.wilkesiana and A.hispida as well as the evaluation of these oils for their toxicity and lavicidal activity.

Materials and methods

Plant material:

Plants were collected at Botanical garden, University of Ibadan; specimens were authenticated at Forestry Research Institute of Nigeria (FRIN) Oyo State. All plants samples were cut in to smaller pieces to facilitate extraction of essential oils from the cell walls of the leaves and this was subjected to hydro distillation for 4h in an all glass Clevenger hydro-distillation apparatus.

GC-MS analysis:

The oil samples was analysed by GC-MS on an Agilent Technologies system consisting of a model 6890N Gas Chromatograph coupled to a Mass Selective Detector (MSD); MS-5973-634071 series and an Agilent Chemstation data system. The GC was fitted with a 30 m x 320 µm x 1 µm film thickness capillary column type coated with DB-1MS equivalent to DB-5. The carrier gas was helium at constant flow rate of 1.0 ml/min, average velocity of 37 cm/s and the pressure was 0.78 psi. The initial column temperature was set at 100 ^{o}C (held for 2 min) to the final temperature at the rate of 5 ^{o}C /min; volume injected was 0.1µL of the diluted oil sample.

Identification of the volatile oil constituents

The individual components of the oils were identified on the basis of their retention indices determined with a reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns with data previously reported in literature [2,16,18].

Toxicity Assay

Brine shrimp lethality test:

Sea water was collected from ocean in Lagos State, South-west Nigeria. Sea water was put in a hatching chamber and Artemia salina eggs were added. The hatching chamber was a plastic bowl, partitioned into two compartments. The partition was perforated such that the nauplii could swim through to the

Tele:
E-mail addresses: sa.aboaba@mail.ui.edu.ng

other side after hatching. The eggs were allowed to hatch for 48 h and mature as nauplii at room temperature. The nauplii were then harvested with a pipette after attracting them to one side of the vessel with a torch light source.

The essential oils were prepared in sea water. 20mg of the test compounds were dissolved in 2 mL of DMSO (dimethylsulfoxide) since they are insoluble in water. Three different concentrations (1000ppm, 100ppm, 10ppm) were prepared in triplicate. 0.5 ml of each of the dose levels was put in a test tube to which 4 ml of sea water was added. Ten shrimps were added to each test tube for each concentration and made up to 5 mL to make 1000 -10 ppm final concentration of the extract. The numbers of survivors were recorded after 24 h.

The concentration killing fifty percent of the larvae (LC_{50}) was determined using the Finney probit computer programme [12]. A control was also set up.

Mosquito rearing: The larva of Anophelis gambiae was collected from stagnant dirty water early in the morning; it was taken to Entomology unit in Zoology Department, University of Ibadan. The larva was separated into the various stages from the pupa. After 24hrs, the pupa had emerged into adult, also the larva has moved to higher stage. The adult were transferred into the netted cages (333x 33x33 cm^3) with a 32 x 32 mesh at 25 + 2^oC and 80 + 2% relative humidity. They were fed with 10% sucrose solution for three days for them to have strength to bite. The 3rd instar larva were reared under the above conditions and were fed continuously mammals blood until pupation.

Mosquito larvicidal assay: Initially, 3rd instar larva were exposed to a wide range of test concentrations and a control to find out the activity range of the essential oils under test (5 to 500ppm). After determining the mortality of larva in this wide range of concentrations, narrower range concentrations, yielding between 10% and 95% mortality in 24 h was used to determine LC_{50} values. Aliquots of the essential oils were placed in a 50 mL netted disposable plastic cup, dissolved in DMSO and made up to 50 mL with borehole water Batches of twenty 3rd instars larvae are transferred by means of droppers to small disposable cup; Two replicates were set up for each concentration and an equal number of controls were set up simultaneously with borehole water. The test containers are held at 25–28 ^oC and a photoperiod of 12 hrs light followed by 12 hrs dark (12L:12D). After 24 hrs exposure, larva mortality was recorded. Moribund larva were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction [23].The LC_{50} and LC_{95} were carried out using statistical analysis software where the slope and heterogeneity analysis are also noted.

A total of 18 compounds and 14 compounds were identified in the oils (0.06% and 0.11% yield, w/w), representing 97.26% and 99.91% of the essential constituents. The compounds detected and their percentage compositions are reported in Table 1.Essential oil from *A .hispisida* contained three monoterpenoids (citral (12.87%), neral (11.04%), nonanal (5.20%)), three sesquiterpenoids (geranylacetone (3.41%), z-α-bisabolene (3.25%) and 6,10,14,trimethyl-2-pentadecanone (13.43%)), one fatty acid (n-hexadecanoic acid (14.69%)). Furthermore, *A.wikesiana* volatile oils consisted of three monoterpenoids (citral (36.10%), neral (30.66% and geraniol (2.45%)).

The brine shrimp lethality of essential oil of *Acalypha hispida* and *Acalypha wilkesiana* showed that the oil from the leaves of were toxic (122.28 and 212.5 µg/ml) respectively. The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant oil bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumour properties [8].

Mosquito larvicidal assay also reveal that *A. hispida*, and *A. wilkesiana* larvicidal has a moderate activity, (LC_{50} 125 and 83.33 µg/ml respectively). The essential oil extracts could be exploited for use in potable waters against mosquito larvae. Field trials are needed to access the efficacy and cost-effectiveness, the source constraint may not allow their practical utility in larger breeding habitat, however, the plant source may be utilised by local people for controlling mosquito larvae in small breeding places like water coolers, tree holes, abandoned wells, drums and containers in and around the rural/suburban dwellings. Such practice would not only reduce the chemical burden on the environment but also promote sustainable utilisation of locally available bio resource by rural communities.

Conclusion

The essential oil composition of the two studied plants revealed the presence of 18 and 14 compounds in *A. hispida* and *A. wilkesiana* respectively. Oxygenated monoterpenoids, sesquiterpenoids and diterpenoids constituted the essential oils, notable were also the presence of higher hydrocarbons.

The result obtained from brine shrimp lethality test is an indication of the toxicity level of the essential oils while the mosquito larvicidal assay has shown the possibility of the oils to be used in potable waters to kill mosquito larvae; this is rather cost effective rather than combating a population of adult mosquitoes.

Acknowledgement:

Financial support of Mc Arthur foundation through the start-up research grant for female faculty is gratefully acknowledged.

References

1. Aboaba, S., Aiyelaagbe, O., Ekundayo, O. (2010) Chemical composition, toxicity and larvicidal activity of the essential oil from the whole plant of *Acalypha segetalis* from South-West Nigeria. Natural Product Communications 5(3), 481-483.
2. Adams, R.P., 2001. Identification of essential oils by ion trap mass spectrometry. Carol Stream, IL. Allured Publishing Corporation.
3. Adesina, S. K., Idowu, O., Ogundaini, A. O., Oladimeji, H., Olugbade, T. A., Onawunmi, G. O., and Pais, M, 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytotheraphy Research, 14: 371-374.
4. Adesina, S. K., Oguntimehin, B. J. and Akinwusi, D. D., 1980. Phytochemical and biological examination of the leaves of *Acalypha wilkesiana* Muell. Arg Quarterly. Journal of Crude Drug Research, 18: 45-48.
5. Adesina, S.K, O. Idowu, A.O. Ogundaina, H.Oladimeji, T.A. Olugbade, G.O. Onawunmi and M. Pais, 2000. Antimicrobial constituents of leaves of *Acalypha wikesiana* and *Acalypha hispida*. Phytother 14: 371-374.
6. Akinde, B. E., 1986. Phytochemical and Microbiological Evaluation of the oils from the Leaves of *Acalypha wilkesiana*. In: Sofowora A, editor. The State of Medicinal Plant Research in Nigeria. University of Ibadan Press, Nigeria, pp. 362-363

Results and Discussion

Table 1 GC-MS analysis of *Acalypha hispida* and *Acalypha wikesiana*

Constituents	RI	*A. hispida* (%)	*A.wilkesiana* (%)
Nonanal	1103	5.20	-
Neral	1235	11.04	30.66
Citral	1240	12.87	36.10
Geraniol	1276	-	2.45
Geranylacetone	1448	3.41	-
z-a-bisabolene	1509	3.25	-
6,10,14,trimethyl-2-pentadecanone	1845	13.43	-
n-hexadecanoic acid	1963	14.69	-
cyclohexadecane	1881	4.28	-
phytol	1949	3.88	-
Tricosane	2300	3.04	-
Tetracosane	2400	5.27	4.38
Pentacosane	2500	4.92	3.50
Hexacosane	2600	-	2.97
Heptacosane	2700	3.22	5.52
2-methyl tricosane	2745	2.96	-
Nonacosane	2900	-	3.23
Triacontane	3000	5.80	5.84
Dicetyl	3200	-	5.26
Total		97.26	99.91
Oxygenated Monoterpenoids		**29.11%**	**69.21%**
Oxygenated Sesquiterpenoid		**22.09%**	-
Oxygenated Diterpenoid		**3.88%**	-
Acyclic Higher Hydrocarbon		**44.45%**	**30.70%**

RT = Retention time, % = Percentage

Alade, P. I. and Irobi, O. N., 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. Journal of Ethnopharmacolgy. 39: 171-174.

7. Aworinde, D.O., Nwoye, D. U., Jayeola, A.O., Olagoke, O., and Ogundele, A. A., 2009. Taxonomic significance of Foliar Epidermis in some members of Euphorbiaceae family in Nigeria. Journal of Botany 4: 17-28.

8. Burkill H, M., 1994. The useful Plants of West Africa. Vol 2 Edition 2. Royal Botanical Garden, Kew, pp 24.

9. Collin, H. and Pareicia, N. L., 1970. Microbiology methods 3rd Edition, London University Press Butterworth.

10. Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistant genes. Science 264: 375-382.

11. Finney, D.J., 1971. Probit Analysis, 3rd Edition, Cambridge University Press, London.

12. Gotep, J. G., Agada, G. O. A., Gbise, D. S. and Chollom, S., 2010. Antimicrobial activity of ethanolic extract of *Acalypha wilkesiana* leaves growing in Jos, Plateau State, Nigeria. Malaysian Journal of Microbiology, 6(2): 69-74.

13. Ikewuchi, J. C. and Ikewuchi, C. C., 2009. Comparative Study of the Mineral Element Composition of Some Common Nigeria Medicinal Plants. The Pacific Journal of Science and Technology, 10(1): 362-366.

14. Ikewuchi, J. C., Anyadiegwu, A., Ugono, E. Y. and Okungbowa, S. O., 2008. Effect of *Acalypha wilkesiana* Muell Arg on Plasma Sodium and Potassium Concentration of Normal Rabbits. Pakistan Journal of Nutrition 7(1): 130-132.

15. Joulain, D. and W.A. Konig., 1998. The atlas of spectral Data of Sesquiterpenes Hydrocarbons. E.B-Verlag Hamburg, Germany.

16. Madziga, H. A., Sanusi, S. and Sandabe, U. K., 2010. Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. Journal of American Science, 6(11): 510-514.

17. Mclafferty, F.W. and D.B. Stauffer., 1989. The Willey/NBS Registry of Mass Spectral Data. John Wiley & Sons, New York.

18. Murugan, K., Murugan, P., Noortheen, A., 2007. Larvicidal and repellant potecial of Albizzia amara Boivin and Ocimum basilicum Linn against dengue vector, Aeded aegypti. Bioresource Technology, 98: 198-201.

19. Onocha, P. A., Oloyede, G. K. and Afolabi, Q. A., 2011. Chemical composition, cytotoxicity and Antimicrobial Activity of Essential Oils of *Acalypha hispida* flowers. International Journal of Pharmacology, 7: 144-148.

20. Santiago, G.M.P., Lemos, T.L.G., Pessoa, O.D.L., Arriaga, A.M.C., Matos, F.J.A., Lima, M.A.S., Santos, H.S., Lima, M.C.L., Barbosa, F.G., Luciano, J.H.S., Silveira, E.R., De Menezes, G.H.A., 2006. Larvicidal activity against *Aedes aegypti* L. of essential oils of *Lipppia* species from Brazil. Natural Product Communications, 1: 573-576.

21. Santos, H.S., Santiago, G.M.P., De Oliveira, J.P.P., Arriaga, A.M.C., Marques, D.D. and Lemos, T.L.G., 2007. Chemical Composition and Larvicidal Activity against Aedes aegypti of essential oils from Croton zehntneri. Natural Product Communications, 4: 473-476.

22. World Health Organisation, 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides.