Chemosensory response of the *Phaseolus vulgaris* L. (Fabaceae) weevil (*Bruchus chinensis* LINNAEUS, 1758 (Coleoptera: Bruchidae)) to *Dioscorea sansibarensis* PAX. (Dioscoreaceae) bulbil essential oil

GODFREY OMARE MAUTI^{1*}, PETER F. KASIGWA¹, JOAN J.E. MUNISSI²,
JUSTUS M. ONGUSO³

¹Department of Zoology and Wildlife Conservation, College of Natural and Applied Sciences University of Dar es Salaam, Dar es Salaam, Tanzania

²Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania

³Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

ABSTRACT. *Dioscorea sansibarensis* bulbils are well known for their toxicity and medicinal applications. In this study, the attraction and mortality of the *Phaseolus vulgaris* weevil (*Bruchus chinensis*) caused by *Dioscorea sansibarensis* bulbil oil was examined in a Completely Randomized Design experiment using a Y-tube olfactometer on a laboratory bench. Statistical analysis at $P < 0.05$ showed 10 μ L had an attraction of 42.67%. In the case of mortality, the results showed that *Phaseolus vulgaris* seeds treated with 40 μ L of the bulbil oil had a harmonic mean of 55.8% mortality of the weevil population, which was higher than with 10 μ L of Actellic 50 EC. GC-MS analysis identified 17 compounds in the bulbil oil, the main constituents of which were 2-pentanone, 4-hydroxy-4-methyl- (17.8%), phenol, 4-ethyl- (11.41%), hexadecanoic acid, methyl ester (11.05%), phenol (7.35%), 9-octadecenoic acid, (E)- (7.2%) and phenol, 4-ethyl-2-methoxy- (7.14%). Attraction by bulbil oil may have been due to the presence of phenolic compounds, and mortality may have been due to hexadecanoic acid, methyl ester and 9-octadecenoic acid, (E)-. Attractants are used for the surveillance and detection of an infestation in stored products. The added advantage of the bulbil oil to cause mortality makes *Dioscorea sansibarensis* bulbil oil useful in the formulation of a botanical pesticide in stored pest management.

KEY WORDS: Attraction, *Bruchus chinensis*, *Dioscorea sansibarensis* bulbil oil, Mortality, GC-MS, Y- tube olfactometer.

* Corresponding author: godiomari@yahoo.com

INTRODUCTION

Bruchus chinensis (LINNAEUS, 1758), also known as *Callosobruchus chinensis* (LINNAEUS, 1758), is a pest species belonging to the Bruchidae family. It causes serious damage to the common bean (*Phaseolus vulgaris* L.) and has a remarkably high rate of reproduction over a short period of time. The female migrates by flying to find better egg laying surfaces (GBAYE et al. 2012). Damage by the *Bruchus chinensis* beetle occurs after the larva hatches from the egg and develops within the seed, where it eats the seed's internal tissue before emerging as an adult and leaving the seed as an empty husk (CHAUBEY 2014).

Plants contain secondary metabolites that, once eaten by some animals, insects and microbial pathogens, lead to their death. These plants produce a scent that acts as an attractant to pollinators and seed-dispersing animals. Such characteristics have given plants different medicinal, antimicrobial and insectivorous properties, and hence, uses (STEVENSON et al. 2017, WINK 2018). Natural products of plant origin in the form of powders, slurries, aqueous/solvent extracts or oils have been tried as insecticides. These plant extracts have secondary metabolites that influence the physiology and behaviour of insect pests involving toxicity, repellency/attraction and anti-feeding effects (UPADHYAY & AHMAD 2011, SALEHI et al. 2014).

Although numerous reports exist on the repellent activities of plant essential oils, fewer data have been published regarding their properties as attractants to *Bruchus chinensis* weevils. Chemical attraction is used in the behavioural control of insects; for instance, the aroma of baker's yeast produced by *Saccharomyces cerevisiae* MEYEN ex E.C. HANSEN and *Kluyveromyces lactis* (DOMBROWSKI) attracts *Bruchus chinensis* and the coffee bean weevil (*Araecerus fasciculatus* (DE GEER, 1775)) (GRAMATICA et al. 1985, YANG et al. 2017).

With the use of Y-tube olfactometry, odours from essential oils retrieved from blueberry (*Vaccinium corybosum* L.), cucumber (*Cucumis sativus* L.), capsicum (*Capsicum annum* L.), chrysanthemum (*Chrysanthemum morifolium* RAMAT.), clove basil (*Ocimum gratissimum* L.) and lavender (*Lavandula angustifolia* L.) were found to attract cranberry weevils (*Anthonomus musculus* SAY, 1831) and raspberry weevils (*Aegorhinus superciliosus* (GUÉRIN-MÉNEVILLE, 1830)) (KOSCHIER et al. 2017, CURKOVIC et al. 2018). HADDI et al. (2018) reported that essential oils recovered from clove (*Syzygium aromaticum* (L.) MERR. & PERRY) and cinnamon (*Cinnamomum verum* J. PRESL) caused both the attraction and death of the bean weevil (*Acanthoscelides obtectus* (SAY, 1831)). A combination of plant oils was found to possess anti-weevil activities, for example, when a mixture of sesame and neem oils applied to mung beans attracted and then killed *Bruchus chinensis* and *Callosobruchus maculatus* (FABRICIUS, 1775) (TALEKAR 1988).

Dioscorea sansibarensis PAX. (Zanzibar yam) is a sun-loving, ornamental climber found along the edges of riparian forests in the coastal regions of Tanzania and Madagascar (BURKILL 1985). The plant produces smooth-surfaced, dark purplish to brown bulbils in the leaf axils, which can reach 6 cm or more in diameter. Toxic and inedible, these bulbils are frequently produced to facilitate the plant's dispersal (WALSH 2009). *D. sansibarensis* is grown by farmers around yam farms in order to persuade potential thieves and monkeys to steal it rather than the main crop of more valuable yams (MAURIN et al. 2016).

When mashed into a paste, the *D. sansibarensis* bulbil has a pleasant and intense aroma. Traditionally, this bulbil has been used for medicinal purposes; of late it has attracted pharmaceutical interest (BURKILL 1985, MAURIN et al. 2016). The bulbil's curative properties are due to the presence of certain bioactive compounds, although there is no scientific rationale to back this up (KUMAR et al. 2017). A literature survey shows that the *D. sansibarensis* plant contains chemical compounds which protect it against bacterial attack and fungi (KUMAR et al. 2017). A study by MAUTI et al. (2019) showed that the leaf oil derived from *D. sansibarensis* repels *Bruchus chinensis* weevils, but no research has yet been conducted on the attractive and insecticidal properties of *Dioscorea sansibarensis* against *Bruchus chinensis*. The present study focused on determining the attractive potency and lethal activity of the essential oil derived from the bulbil of *Dioscorea sansibarensis* against the stored-legume pest *Bruchus chinensis* and on analysing its chemical composition using gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Materials

Healthy dry common bean seeds *Phaseolus vulgaris* were purchased directly from a farm after harvest and stored with 10 ± 2 % moisture content. The Y-tube olfactometers for this work were constructed in the glass-blowing section of the Chemistry Department of the University of Dar es Salaam (UDSM), Tanzania. Acetone (analytical grade) was purchased from Chemical and Equipment Laboratory Suppliers in Tanzania. The commercial synthetic pesticide, Actellic 50 EC (500g/L AI, Syngenta, Switzerland), was purchased from Ubungo Agricultural Input Suppliers in Dar es Salaam for use as the positive control in the mortality test of *Bruchus chinensis*.

Collection and preparation of plant materials

Fresh bulbils of *Dioscorea sansibarensis* were identified and harvested from the Zoology-Botany forest of UDSM (6° 45'0" S, 39° 15'0" E, altitude 80 m a.s.l.). Further

morphological identification was carried out in the herbarium of the Botany Department of UDSM and allocated the voucher reference number FMM 3910b.

Extraction of essential oil

Fresh aerial bulbils of *D. sansibarensis* were collected, washed, rinsed with distilled water and then macerated into small pieces. The pieces were air dried for 5 days at the entomology laboratory of UDSM. After drying, 250 g of bulbils were ground and homogenized to a powder using an electric grinder. The essential oil was extracted from the powdered bulbil for 4 hours by hydro-distillation in a Clevenger apparatus. The oil was concentrated under vacuum at 40 °C and stored in a refrigerator at 4 °C for the bioassays.

Determination of the essential oil components

The identity of the essential oil constituents was established by GC-MS (a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph). The mass selective detector was operated in electron impact mode with an ionization energy of 70 eV and a mass range from m/z 40 - 380. The temperature of the HP-5 column (30 m long and 0.25 mm internal diameter with a film thickness of 0.25 µm) was initially maintained at 60 °C, then gradually increased to 250 °C at a rate of 5 °C /min. Helium (99.9%) was used as the carrier gas. The temperature and its rate of increase were adjusted if peak separation was inadequate. Each sample was dissolved in hexane to give a 1% w/v solution; 0.2 µL of the oil sample was injected at 250 °C into the GC-MS equipment from which the gas chromatograms and mass spectra were obtained. The gas chromatograms were used for the quantitative analysis of the individual constituents. The mass spectra were used for identifying the constituents by matching their spectra to those recorded in the Wiley 275 and NIST Mass Spectral Library with reference to the associated database and literature.

Rearing of test weevils

One kg of dry, undamaged and healthy *Phaseolus vulgaris* beans were dried in an oven at 60 °C for 1 hour. They were placed in glass jars (27 cm height × 16 cm diameter). Approximately 350 adults of the collected *Bruchus chinensis* weevils were released into the jar and the mouth subsequently covered with muslin cloth and secured with a rubber band. The bottles were then incubated in darkness at 28 ± 2 °C and $65 \pm 5\%$ relative humidity for mating and oviposition for 2 weeks. The weevils were then separated from the seeds by sieving and the seeds returned to their container for continued incubation. The F1 progeny aged two weeks were selected for the bioassay.

Chemosensory bioassay

The attractive activity of the bulbil oil on *B. chinensis* was assessed using a Y-shaped olfactometer. The Y-tube was placed in the centre of a black box of dimensions 23 x 16 x 11 cm, lined with black paper in order to avoid visual stimuli produced by the halogen lamp when switched on. The Y-tube olfactometer consisted of a Y-shaped glass tube with three compartments (arms), each 1 cm in diameter and 10 cm long. These compartments were the treatment arm, the control arm and the arm for introducing weevils. The end of each arm was closed with a 3 cm diameter glass stopper. The stoppers had two narrow grooves allowing air to flow into the olfactometer during the bioassays.

Filter paper discs (Whatman No.1, diameter 1 cm and weight 140 mg) were dipped into dilutions of bulbil oil 10, 20, 30 and 40 μL per 1 mL of acetone and allowed to air-dry. Acetone was used to sterilize the filter paper disc that was used as the negative control disc. The discs were inserted into the treatment compartments, after which air-suction through a Tygon tube was applied at the Y-junction by means of an aspirator pump before the introduction of the weevils. This ensured that the olfactometer did not become saturated with the vapour from the oil, which was confined to the treatment arm of the olfactometer. The insects were induced by light and allowed to walk and decide which arm to enter at the bifurcation.

Ten pairs of weevils were selected for each assay and introduced at once into the olfactometer. Advantage was taken of the fact that weevils are negatively phototactic by illuminating the introduction compartment of the weevils into the olfactometer with light from a 60-watt bulb placed 15 cm away and screening the rest of the olfactometer in a cardboard box. An average of 80-85% of the weevils was thus induced to migrate from the introduction arm to either the control or treatment arm during the bioassay. This was run for one hour, after which the numbers of weevils in the control arm (NC) and treatment arm (NT) were counted. The percentage attraction (% PA) was calculated using the formula (source):

$$\% \text{ PA} = \{ (\text{NT} - \text{NC}) / (\text{NT} + \text{NC}) \} \times 100,$$

where % PA is the percentage attraction, NC is the number of weevils in the control arm and NT is the number of weevils in the treatment arm.

Bruchus chinensis mortality test

About 50 g of clean, disinfected, common bean seeds were weighed into sterilized glass bottles. Concentrations of bulbil oil (0.036, 0.072, 0.108 and 0.144 mg/mL) and Actellic 50 EC (0.01, 0.02, 0.03 and 0.04 mg/mL) were made by respectively diluting 10, 20, 30 and 40 μL of oil or Actellic 50 EC in 5 mL of acetone. Each dilution was then mixed with the bean

seeds. Beans sterilized by acetone were used as the negative control. Ten pairs of newly emerged adult weevils were introduced to the treated and untreated bean seeds in the glass bottles and covered with a muslin cloth. Each experiment was replicated four times. The experiment was arranged in a Completely Randomized Design on a laboratory bench. The number of dead insects were recorded every 24 hours for 5 days. Percentage mortality was computed using the method adopted by ABBOTT (1925).

Statistical analyses

All recorded data on weevil attraction and mortality were converted to percentages and the means compared by Duncan's New Multiple Range Test (DMRT) using the statistical programme Minitab 2017 version 18. ANOVA was used to determine the differences in the effectiveness of bulbil oil at different concentrations using SPSS software Version 20, including a normality test, homogeneity distribution and post-hoc analysis utilizing the Tukey HSD test at $P \leq 0.05$. The independent variables in this model were the treatment doses and the duration of exposure, while the dependent variables were the percentage attraction and mortality.

Table 1. Chemical composition of the essential oil extracted from the bulbil of *Dioscorea sansibarensis*.

No.	Constituents	MM ^a	RT ^b	% ^c
1.	2-Pentanone, 4-hydroxy-4-methyl-	116	5.05	17.8
2.	Phenol	94	10.08	7.35
3.	2-Octanol, (S)-	130	10.47	1.51
4.	Phenol, 2-methyl-	108	12.40	1.60
5.	<i>p</i> -Cresol	108	13.07	4.67
6.	Phenol, 2-methoxy-	124	13.28	5.39
7.	Phenol, 2-ethyl-	122	15.00	2.80
8.	Phenol, 2,4-dimethyl-	122	15.27	1.25
9.	Phenol, 4-ethyl-	122	15.86	11.41
10.	Benzenepropanenitrile	131	17.82	3.80
11.	Phenol, 4-ethyl-2-methoxy-	152	18.93	7.14
12.	Indole	117	19.38	6.21
13.	Hexadecanoic acid, methyl ester	270	33.87	11.05
14.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	37.03	4.35
15.	6-Octadecenoic acid, methyl ester, (Z)-	296	37.15	3.95
16.	9-Octadecenoic acid, (E)-	282	37.96	7.20
17.	1-Heptacosanol	396	43.64	2.53

^a Molar mass (g/mol) ^b Retention time (minute); ^c Percentage composition of a compound.

RESULTS

Constituents of essential oils

Dioscorea sansibarensis bulbils yielded $0.43 \pm 0.03\%$ (w/w) of oil with a density of 0.36 ± 0.05 g/mL. GC-MS analysis revealed a total of 17 compounds eluted in the GC-MS analyser during 45 minutes. The main components and their percentage compositions were 2-pentanone, 4-hydroxy-4-methyl- (17.8%); phenol, 4-ethyl- (11.41%); hexadecanoic acid, methyl ester (11.05%); phenol (7.35%); 9-octadecenoic acid, (*E*)- (7.2%) and phenol, 4-ethyl-2-methoxy- (7.14%) (Table 1 and Fig. 1). The bioassay results showed that the bulbil oil acted as an attractant to *Bruchus chinensis*.

Chemosensory bioassay using the Y-tube olfactometer

In this study, *Dioscorea sansibarensis* bulbil oil attracted *Bruchus chinensis* weevils. The 30 μ L dilution of bulbil oil exhibited an attraction of 33.33% as compared to the 10 μ L dilution (42.70%) (Fig. 2). The sample size ($N = 200$) had a normal distribution: Levene's test revealed that the data were heterogeneously distributed at $L(7, 192) = 9.143$; $P < 0.05$. Tests of within-subject effects showed that the oil treatments influenced 91.2% ($R^2 = 0.912$) of the total weevil attraction at $F=283.283$; $P < 0.05$. Statistically, the 10 μ L dilution of bulbil oil had a higher attraction activity than the 20 μ L or 30 μ L dilutions.

Mortality test

The tabulated results show that on the first day of weevil exposure to the common beans treated with different concentrations of bulbil oil and Actellic 50 EC, 20 μ L of bulbil oil elicited a higher mortality rate than 10 μ L of Actellic 50 EC (Table 2). The data ($N = 640$) were subjected to statistical analysis. The Shapiro-Wilk test showed a normal distribution of the data as Levene's test recorded a heterogeneous distribution at ($F(39,600) = 24.256$; $P < 0.05$). Testing the between-subject effect showed that bulbil oil influenced 88.6% ($R^2 = 0.886$) of the results on mortality. Statistically, 30 μ L of bulbil oil with a harmonic mean of 38.72% had a homogeneous mortality activity equivalent to 10 μ L of Actellic 50 EC, which had a harmonic mean of 42.13%. The results showed that 40 μ L of bulbil oil with a harmonic mean of 55.8% elicited a higher mortality than 10 μ L of Actellic 50 EC.

Table 2. Mortality of *Bruchus chinensis* weevils reared for 5 days on *Phaseolus vulgaris* seeds treated with different concentrations of *Dioscorea sansibarensis* bulbil oil and Actellic 50 EC. Conc. – concentration [$\mu\text{l}/50\text{g}$].

Treatment	Conc.	Mean % mortality at days after post-treatment $\pm$ S.E				
		Day 1	Day 2	Day 3	Day 4	Day 5
Bulbil oil	10	0.00 $\pm$ 0.00	2.92 $\pm$ 1.72	5.81 $\pm$ 2.27	16.04 $\pm$ 4.73	25.10 $\pm$ 8.86
	20	9.02 $\pm$ 1.16	13.56 $\pm$ 1.59	28.57 $\pm$ 4.34	33.33 $\pm$ 4.36	45.05 $\pm$ 7.35
	30 ^a	11.45 $\pm$ 3.64	21.19 $\pm$ 4.21	29.77 $\pm$ 4.45	56.22 $\pm$ 6.91	74.97 $\pm$ 6.79
	40	26.86 $\pm$ 6.14	43.89 $\pm$ 6.14	48.73 $\pm$ 5.24	70.22 $\pm$ 3.48	89.33 $\pm$ 5.23
	10 ^a	5.14 $\pm$ 2.05	15.75 $\pm$ 3.62	39.66 $\pm$ 5.35	60.38 $\pm$ 3.96	89.74 $\pm$ 4.02
Actellic 50 EC	20	67.80 $\pm$ 3.75	90.46 $\pm$ 3.75	97.06 $\pm$ 2.94	100.00 $\pm$ 0.00	100.0 $\pm$ 0.00
	30	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
	40	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Untreated	0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	10	0.00 $\pm$ 0.00	2.92 $\pm$ 1.72	5.81 $\pm$ 2.27	16.04 $\pm$ 4.73	25.10 $\pm$ 8.86

Treatments with the same letters in the table are not statistically significantly different from each other. (N = 640), ($P < 0.05$) using Tukey's HSD test.

DISCUSSION

Most research on fumigant insecticides focuses strongly on their repellent properties. Fewer studies have been carried out on the attraction potentiality of plant-based insecticides. The present one used Y-tube olfactometry to demonstrate that bulbil oil attracted *Bruchus chinensis*. Similarly, *Bruchus chinensis* weevils were attracted to oils of the water hyacinth (*Eichhornia crassipes* (MART.) SOLMS) (RANI & JAMIL 1990). CHILUWAL et al. (2017) likewise found attraction properties expressed by essential oils of *Illicium verum* HOOK.f., *Bursera delpechiana* POISS. ex ENGL., *Croton anisatum* H.BN., *Cinnamomum cassia* (L.) J. PRESL and *Aniba rosaeodora* DUCKE to *Bruchus chinensis* using Y-tube olfactometry.

It was also found that high levels of both attraction and mortality of *B. chinensis* were obtained with the highest dose (40 μL) of the bulbil oil. This was due to the high concentration of the active compounds from the zone of application. This upholds the findings on essential oils of different doses and various concentrations on the attraction of Coleopterans where a higher dosage increases attraction (NTONIFOR et al. 2010). CHILUWAL et al. (2018) showed that the highest dose of essential oil from *Gaultheria fragrantissima* WALL. was found to attract many individuals of *Bruchus chinensis*.

In this study, attraction by bulbil oil could have been induced by the high content of phenolic compounds present in bulbil oil. This is supported by OWAGA et al. (1988), who

reported that the presence of phenolic compounds was the major attractant of tsetse flies to buffalo urine. It was also reported that phenolic compounds applied to pumpkins elicited pheromone activity in the melon fruit fly (*Bactrocera cucurbitae* (COQUILLET, 1849)) (SHARMA & SOHAL 2016). The phenolic compound p-cresol was found to attract *Toxorhynchites* THEOBALD, 1901 species (LINLEY 1989, NCHEKWUBE & UZOH 2017). Furthermore, the phenolic compounds of coriander (*Coriandrum sativum* L.) and false fennel (*Aneth graveolens* L.) attracted and killed the wax moth (*Galleria mellonella* (LINNAEUS, 1758)) (OULEBSIR-MOHANDKACI et al. 2018). CHILUWAL et al. (2018) reported that the phenolic compounds in *Gaultheria fragrantissima* essential oil have been found to cause pheromones activity to *Bruchus chinensis*.

The *B. chinensis* weevils attracted to the treated *Phaseolus vulgaris* beans were killed. This was similar to the report by HADDI et al. (2018), where clove and cinnamon essential oil, applied to kidney beans and cranberries, attracted and killed the bean weevil *Acanthoscelides obtectus*. In this study, weevil mortality may have been enhanced by the presence in the bulbil oil of hexadecanoic methyl ester, 9-octadecenoic acid, (E)- and p-cresol. The literature reports that hexadecanoic methyl ester and 9-octadecenoic acid, (E)- possess pesticidal and insectifuge activity (TAYADE et al. 2013, SHIBULA & VELAVAN 2015). Essential oil of *Ocimum basilicum* L., with a high concentration of hexadecanoic methyl ester as one of its major components, exhibited insecticidal activity against *Bruchus chinensis* (REGNAULT-ROGER 1997). 9-octadecenoic acid, (E)- derived from *Moringa oleifera* LAM. and *Cinnamomum aromaticum* (L.) J. PRESL caused mortality in *Bruchus maculatus* and *Callosobruchus chinensis* (ISLAM et al. 2009, CUI et al. 2017, AJAY et al. 2018). The phenolic compound p-cresol may have increased weevil mortality owing to its pesticidal and antifeedant properties (MARCUS & LICHTENSTEIN 1979, NCHEKWUBE 2017). Weevil mortality by bulbil oil may have been enhanced by the combination of lethal compounds that increased its toxicity to the target weevils.

CONCLUSION

Attractants are used for the surveillance and detection of infestations in stored products. Furthermore, the ability to kill the weevil is an added advantage in the control of storage pests. The findings of this study indicate the potential of *Dioscorea sansibarensis* bulbil oil for use in a formulation as an attractant in bean weevil management. Although this research has demonstrated the scientific uses of *D. sansibarensis* bulbil as a *Bruchus chinensis* attractant and as an antifeedant, further investigation of the mechanism of action of bioactive compounds extracted from the bulbil of *Dioscorea sansibarensis* is recommended.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to DAAD-NAPRECA for the study scholarship [Reference number 91560175] for the research topic “Studies on the antifeedant properties of *Dioscorea sansibarensis* (PAX.) on *Bruchus chinensis* L. in the protection of stored *Phaseolus vulgaris* L. beans”. We are grateful to Mr. Frank MBAGO of the Botany Departmental Herbarium for identifying the *Dioscorea sansibarensis* plant material, and to Dr. Fortunatus SUNGHWI of the Chemistry Department, University of Dar es Salaam, for facilitating the identification of *Dioscorea* leaf essential oil constituents, retention index and chemical composition by GC-MS.

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Received: 11 February 2019

Accepted: 24 April 2019