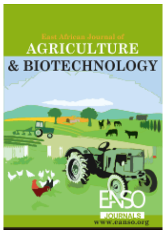

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Original Article

Repellence Of Volatiles And Extracts Of *Solanecio Manii* To Subterranean Termites, *Macrotermes Natalensis* In Laboratory Test

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ABSTRACT

Plant based pesticides have been touted as alternatives to supplement synthetic pesticides for integrated pest management as they pose little threat to health and environment hence a need for affordable and eco-friendly alternative pesticides of botanical origin. *Solanecio manii* plant used traditionally by communities in Kericho to repel insects from dwellings was evaluated for repellence potential against *Macroteremes natalensis* termites. Samples of S. *manii* roots stem and leaves were collected and prepared appropriately for each test as fresh, dried or crushed forms. The dried plant parts were extracted using methanol and hexane and extracts prepared to 2, 4, 6 and 8 mg/ml dilutions respectively. Termite repellence was evaluated in a dual choice Y-tube olfactometer in a choice test and in petri dishes with inoculated paper discs (10x10) mm and rings of 10 mm width. Results indicated that on average, the highest and lowest percentages of termites contacted filter papers discs treated with pure water (23.5%) and 8mg/ml of leaves phytochemicals (2.4%) respectively. 6.6% of termites was the lowest recorded in the test compartment when fresh crushed leaves were placed in the test compartments of the Y-tube olfactometer. The average number of termites that contacted filter paper discs on repellence was significantly higher when filter paper was treated by the control than when treated with *S. manii* phytochemicals extracted using hexane ($p < 0.001$) and methanol ($p < 0.001$) solvents. The study concludes that *S. manii* extracts are potential repellents to subterranean termites authenticating and scientifically justifying traditional knowledge that exists regarding use of *S. manii* for termite control. Therefore, enhanced use of the plant extracts as botanical pesticide friendly to the environment is recommended.

INTRODUCTION

For many years, mankind has used plant materials as curative, food and poverty alleviating resource because they contain many useful bioactive chemical compounds (Masinde, 1996; Jacobson, 1989). These bioactive plant compounds include alkaloids, steroids, tannins, glycosides, volatile oils, resins, fatty acids, phenols and flavonoids stored in specific plant parts such as leaves, flowers, bark, seeds, fruits, roots and stem (Gaya, Kawaka, Muchugi & Ngeranwa, 2013). The beneficial pesticides, repellents and pharmaceutical products derived from plant materials comprise a combination of these secondary products (Tonthubthimthong, Chuaprasert, Douglas, & Luewisutthichat, 2001) and considered safe to use and friendly to the environment.

Termites being a problem to trees and agricultural crops have to be managed effectively and therefore need an integrated combination of tactics that controls them on desired environment in order to prevent attack. The possible strategy would be to use termite repellents products and or termite-repellent plant parts (Verma, Satyawati, & Rajendra, 2009). The use of termite repellent plants and their integration with other control measures could be practical in providing economic ways of controlling them (Upadhyay, Ramalakshmi, & Rao, 2010; Prisila, Mtei, & Ndakidemi, 2014). Indeed, plant-based pest control agents have been touted as alternatives to synthetic chemicals for integrated pest management. Such phytochemicals reputedly pose little threat to environment or to human health (Upadhyay, Ramalakshmi, & Rao, 2010).

Bioactivity of plant-based compounds is a subject of increasing importance as an approach for insect control using chemicals of plant origin. Even though it has been studied at laboratory level extensively, only a handful of the compounds are currently being used in agro-systems and forestry (Sileshi, Nyeko, Nkunika, Sekamatte, Akinnifesi, & Ajayi, 2009). According to Koul (2008), the known active plant-based repellents belong to groups like chromenes, polyacetylenes, saponins, quassinoids, cucurbitacins, cyclopropanoid acids, phenolics, flavonoids, alkaloids, various types of terpenes and their derivatives but a few highly active ones have been looked into from a commercial point of view. Tree crops are known to be bulky with long rotations and low financial gains compared with agricultural crops so that the use of synthetic chemicals is not only difficult but expensive to formulate and toxic to human beings (Soomro, Seehar, Bhangar, & Channa, 2008). This called for the use of integrated pest management strategies (IPM) as a basis of pest control thereby calling for the introduction of biological control and use of fast-growing repellent plants (Sileshi, & Katanga, 2012). This study therefore seeks to evaluate repellence of *S. manii* volatiles and extracts to the subterranean termites, *M. natalensis.*

MATERIALS AND METHOD

Plant material collection and preparation

S. manii (Fig.1) was first identified and a specimen deposited at Department of Botany University of Kabianga. Mature leaves of *S. manii* were randomly selected, collected, packaged, labelled and processed as and when required for each experiment.

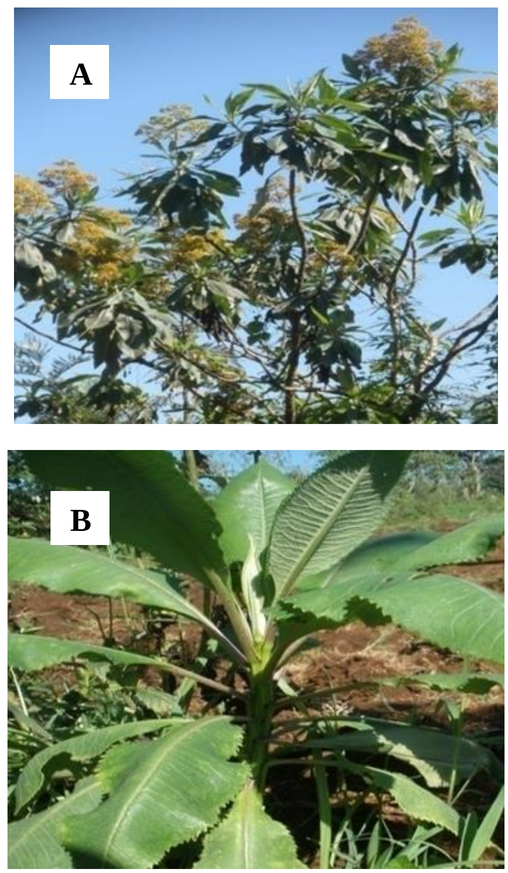


Figure 1: ***Solanecio manii* (A) young and (B) mature plant with flowers.**

Solvent extraction of *S. Manii* leaves

Air dried leaves of *S. Manii* were grounded in a hammer mill to fine powder. Extraction with methanol and hexane was carried out by soaking 50 grams of *S. manii* powder in 250 ml solvent in sealed air-tight beakers then shaken continuously for 48 hours in an electric shaker. The solvent with extracts was then filtered and filtrate evaporated in vacuum rotor vapour (Gupta, Naraniwal, & Kothari, 2012). Dry *S. manii* extracts free from solvent where then stored in air tight glass vials at 4^{o} C awaiting use.

Termite Collection

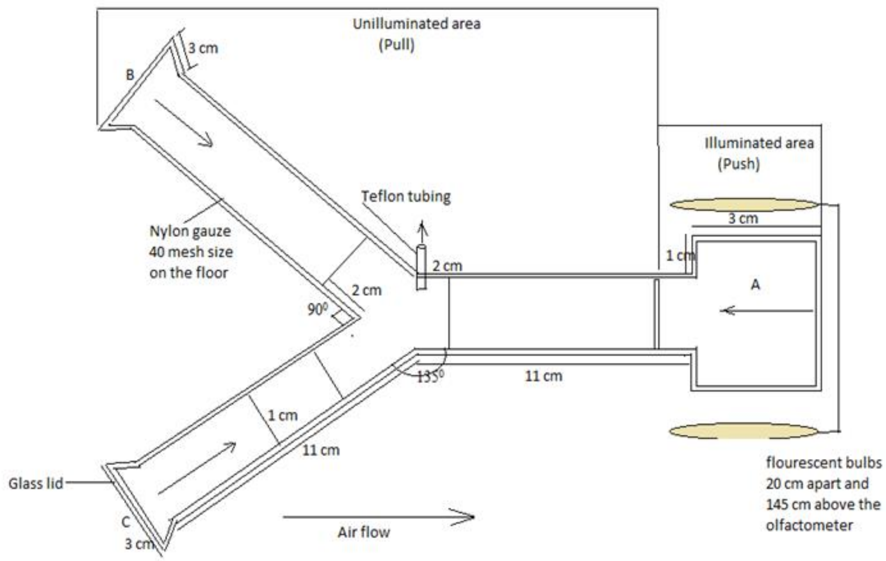
Subterranean termites *Macrotermes natalensis* were trapped from identified termite mounds located within the University of Kabianga (1,739m above sea level, 00^{o} 26.930' South, 035^{o} 08.236' East) according to a modified method described by (Tamashiro, Fijii, & Lai, 1973; Gitonga *et al.*, 1995) as follows: Subterranean termite mound was dug up, nests containing termites neatly removed, covered using dark polythene sheets, placed in a bucket with moistened cotton wool to maintain the required moisture levels and transported to a laboratory maintained at temperature of $24^{o} – 26^{o}$ C and relative humidity of 76 %. Pieces of wood were introduced into the bucket together with a little soil from the mound to attract termites out of the nest. A sample of twenty termites were then collected, anaesthetized in absolute alcohol, mounted and observed under dissecting and compound microscope for subsequent identification using the taxonomic key as described by (Gay, 1979; Scheffrahn, & Su, 1995).

Treatment of Filter Papers With *S.manii* Extracts

S. manii extracts were diluted to series concentrations of 0, 2, 4, 6 and 8 $mg/ml_{w/v}$ in hexane, methanol and control in distilled water. Filter papers (10x10mm discs and 10mm width 72.5 mm internal ϕ rings) were immersed separately in *S. manii* extract solutions for 12 hours. Initial weight of filter papers without extracts was measured before immersion (W_1). After treatment, filter papers were then dried to constant weights and final weights measured (W_2).

Setup of Dual Choice Y-Tube Olfactometer

A dual choice Y-tube olfactometer constructed from glass and consisting of three compartments (A, B and C) designed by Mburu *et al.* (2009) was used to test the repellence of *S. manii* leaves to termites (Fig. 2). The first compartment (A) is the termite release site, the second and third compartments (B and C) being the source of test and control treatments respectively. On the upper part of the junction where the arms of the three compartments of the olfactometer meets, there is an outlet of teflon tubing mounted to a respirator pump to facilitate air movement in the olfactometer. Air from the compartments meet at the junction and is sucked out through the teflon tubing by the respirator pump. A flow meter is connected to the olfactometer to regulate the amount of air entering the test and control compartments to ensure that the same amount of gas is pumped in. Two conical flasks each 100ml were used for holding test materials. One held the plant materials as the source for volatiles while the one used as a control was lined with moist filter paper to supply fresh humid air. The floor of the test and control compartments of the olfactometer was lined with a nylon gauze mesh wire to provide traction for smooth movement of termites. The arms of the olfactometer were covered with a dark cotton cloth to cut off light as an inducement for termites to move from the release compartment and move along the runway and make a choice at the junction between the two arms. The combination of brightness at the release compartment and darkness inside the olfactometer acted as a push-pull set of visual stimuli. Observation of the movement of termites in the compartments was determined to assess whether the plant extract is repellent depending on the number of termites that follow a given arm as observed at intervals of 10 minutes for 20 minutes when the black cotton cloth is removed for observation and subsequent recording.



A is the termite release chamber B & C are the test and control chambers respectively.

Figure 2: Diagram of the Y-tube olfactometer.

Choice termite repellence test against *S. manii* volatiles

This test was based on a dual choice Y-tube olfactometer (Fig 2). Termites were picked individually using soft forceps and placed in 9 cm diameter petri dishes lined with moist filter paper (Whatman paper no. 1ϕ9 cm). Petri dishes with termites were then kept in a room set at 25^{o} C ± 1^{o} C and a Relative Humidity of 76 % ± 2 % in darkness for 20 minutes to acclimatize. A total of 20 termite workers and five soldiers for social cohesion were then randomly selected from the stock population and introduced into the release compartment (A) of the dual choice Y-tube olfactometer. The test plant materials were introduced into the test compartment B and control material into compartment C separately and replicated three times. Individual termites were counted as they moved within the olfactometer's arena at introduction and at time intervals of 10 and 20 minutes then evaluated as the % of the initial number. After testing multiple termites around the arena pseudo-randomized each time with the arrangement of olfactory stimuli, it was possible to determine which olfactory stimuli was most preferred and the one avoided. Air flow rate was checked regularly, ensuring that it was 10 ml/min through each arm.

Choice Termite Repellence Test against *S. manii* volatiles

Choice termite repellence test against *S manni* plant materials was carried out as follows: Discs (10x10) mm and rings of 10 mm width, 72.5 mm internal ϕ were cut from dry whatman filter papers previously treated to 2, 4, 6 and 8 mg/ml of plant extracts (Rehman *et al*. 2005). The discs and rings were then placed at the center and the edges of petri dishes respectively with untreated ones as control. To maintain humidity in the samples, 0.2 ml of distilled water was added periodically to moisten the paper discs. Twenty (20) worker termites and 5 soldiers were randomly selected from the stock population and introduced into the petri dishes. This was replicated thrice for each set of treatment and control. A total of twenty-four treatments and three positive control units were arranged in CRD and replicated three times to make a total of eighty-one. The number of termites making contact with each paper disc was recorded at 5 minutes intervals during the first 30 minutes then at 10 minutes intervals for the next 30.

Chemical Analysis of Leave Extracts

One gram of well ground *S manni* was extracted using 10 ml of methanol and hexane differently and filtered using whatman paper no. 1 and the filtrate kept at 4° C. The filtrate was then concentrated in a rotary evaporator to approximately 1 cm^3 and reduced further to 0.5 cm^3 under a fine stream of nitrogen gas. The yield extracts were run in Agilent gas chromatograph model 7890P equipped with Agilent mass selective detector model 5977A (GC-MS).

The GC-MS operating conditions were; Oven temperature was maintained initially at 70° C for one minute, increased at 15° C / minute to 175° C, then at 2° C / minute to 215° C, at 10° C / minute to 265° C and finally at 20° C / minute to 290° C and held for 8 minutes. Injection volume was 1 µl, injected in split less mode at injection temperature of 250° C. Helium gas was used as the carrier gas at a flow rate of 1 cm^3/minute in a RTX-5SIL MS (Restek) (30M X 0.25 mm id., 0.25 µm film thickness) fused - silica capillary column interfaced by spectral library search to know the actual compound (Nanyonga, Opoku, Lewu, Oyedeji, & Singh, 2013). Chemical composition, antioxidant activity and cytotoxicity of the essential oils of the leaves and stem of Tarchonanthus camphoratus. Peaks were obtained from analytical instrument for qualitative analysis.

RESULTS AND DISCUSSIONS

Repellence of *S. manii* leaves to subterranean termites

The average percentage of termites in the release, test and control compartments of Y-tube olfactometer subjected to fresh, dry and powder forms of *S. manii* leaves are presented in table 1.

Table 1: Average % of Termites in the Y-tube Olfactometer Compartments

Condition of *S. manii* leaves	Time interval	Average % Termites in Compartments: Release	Test	Control
	10	32.6	29.8	37.6
None	20	27.6	32.8	39.6
	10	43.8	20.8	35.4
Fresh uncrushed	20	60.2	8.5	31.3
	10	42.9	21.4	35.7
Fresh crushed	20	66.7	6.6	26.7
	10	45	10	45
1 day air dried	20	53.3	6.7	40
	10	35	10	55
3 days air dried	20	36.3	8.3	55.4
1 day air dried	10	37.6	14.1	48.3
powdered	20	26.7	6.7	66.6
3 days air dried	10	35	15	50
powdered	20	37.6	11.5	50.9

Termites distribution ranged from 32.8% - 39.6% in test and control chambers respectively after 20 minutes of release in the absence of *S. manii* leaves. Introduction of fresh crushed *S. manii* leaves in the test compartment reduced the distribution of termites to a range of 6.6%- 26.7% in test and control chambers with 66.7% returning back to release chamber. Generally, on release termites tend to move to either the test or control compartments of the Y-tube Olfactometer with time in all the tests. However, they made a choice at the Y-junction depending on the repellence of the test material and either chose to return back to the release chamber or move to the control chamber. The number of termites that visited the test compartment however reduced due to repellence of the volatiles emanating from *S. manii* test sample. Volatiles in fresh leaves tend to escape after harvesting and crushing justifying the use of fresh leaves by local people to rid termites from their premises.

To determine the variation in termite repellence of test samples, a (7x2x2x3) factorial analysis was carried out on various forms of *S. manii*, time interval and number of termites in the release, test and control compartments of Y-tube olfactometer respectively. The results in table 2 indicates that the mean number of termites varied significantly based on the condition of the leaves (A), time interval (B) and compartment (D), but not on swop (C) which is the change of compartment and control positions.

Table 2: Summary of factorial tests of leaves condition on repellence

Dependent Variable: No of termites observed in compartment	Sig.
Source	
Leaf condition (A)	0.049
Time interval (B)	0.000
Swop (C)	0.951
Compartments (D)	0.000
A * B	0.475
A * C	0.997
A * D	0.080
B * C	0.583
B * D	0.075
C * D	0.410
A * B * C	0.994
A * B * D	0.091
A * C * D	0.187
B * C *	0.811
A * B * C * D	0.877

The *p* value for the main effects of leaves condition, time interval, swop and compartments are p=0.049, < 0.001, 0.951 and < 0.001 respectively. Tukey HSD indicated that the mean number of termites observed after 10 minutes are significantly higher than the mean number of termites observed after 20 minutes (p = < 0.001). However, swopping the compartments had no significant effect on the ability of leaves to repel subterranean termites. In addition, there was no significant interaction between all the four factors under investigation (p> 0.05).

Multiple comparison results of Post hoc analysis using Tukey HSD at different levels of *S. manii* leave conditions and % termites in release, test and control compartments indicated that the mean number of termites was significantly higher in the control compartment than in the release and test compartments when either fresh uncrushed leaves (p = 0.041), fresh crushed leaves (p = 0.033), 1 day air-dried leaves (p = .045), 2 day air-dried leaves (p = .018), air-dried unpowdered leaves (p =0.024), or air-dried powdered leaves (p = 0.049) in the test compartment. Therefore, regardless of the condition, leaves from *S. manii* are effective repellents to *M. natalensis* termites, suggesting presence of a volatile compound that has repellence effects.

Introducing *S. manii* leaves at whatever form in the test compartments led to a significant reduction in the number of termites entering the test compartment. Air drying or grinding plant parts does not influence the amount, composition and concentration of plant phytochemicals (Azwanida, 2015; Hesse, 2002; Fattorusso and Taglialatela-Scafati, 2008).

Choice Repellence Test of *S. Manii* Leaves to Subterranean Termites

The average % of termites making contact with paper discs impregnated with hexane and methanol extracts of *S. manii* after one hour are reported in Figure 4 below.

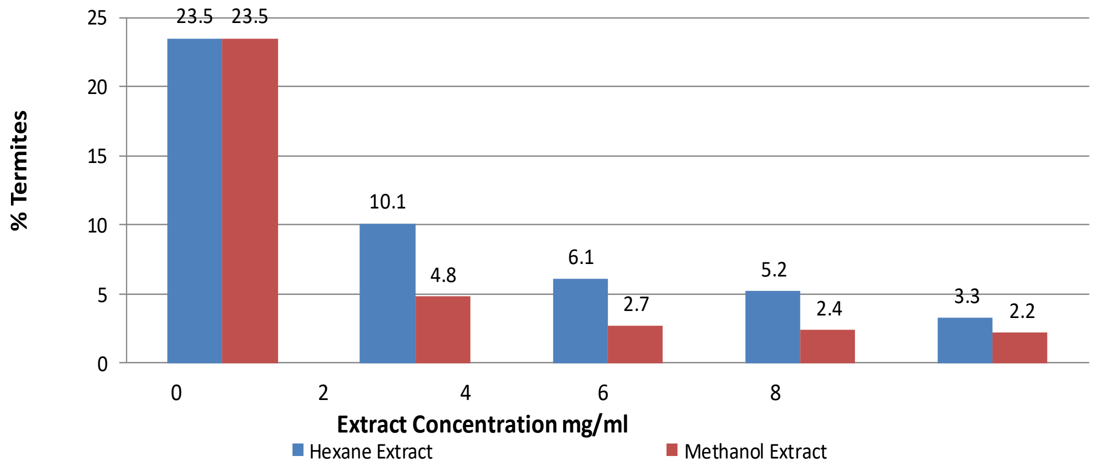


Figure 4: % termites that contacted filter papers disks treated with *S. manii* extracts.

Fig. 4 shows the average percentage of termites that contacted the filter paper discs treated with different concentrations of *S. manii* leave extracts. The highest percentage of termites (23.5 %), made contact with untreated filter papers, however this decreased with increasing extract concentration. Factorial analysis indicates that the % of termites contacting the paper discs varied significantly with the type of extract and its concentration. In all cases, methanol extract exhibited higher repellence than ethanol

Any substance that is able to keep pests away from the source of food without killing them is a repellent (Aswathi, 2007). Therefore, this study's findings that *S. manii*'s extracts using either methanol or hexane solvent kept away more termites from reaching treated filter paper discs which was a source of food more than the control means that such extracts are termite repellent. This concurs with Dodia *et al*. (2008) that repellence success can only be achieved with plant compounds having bad odour like *S. manii*. This is also in agreement with observations by Nnamonu and Onekutu (2015) that little is known about plant phytochemicals that are repellent to insects as only a few plants have been investigated.

The differences in repellence abilities from *S. manii's* extracts from methanol and hexane may be explained by Anees *et al*. (2009). Indeed, methanol and hexane have been used as plant compound extraction solvents for long, however the two solvents extract different plant compounds (Anees *et al*. (2009; Sasidharan *et al*., 2011). Khoddami *et al*. (2013) observed that while methanol solvents extract polar compounds, hexane solvents removed non-polar plant compounds. Therefore, it is concluded that polar *S. manii* plant extracts are effective repellents against termites compared to non-polar ones.

The findings that extracts from the leaves, of *S. manii* can repel subterranean termites concur with Gaya *et al*. (2013), Blaske *et al*. (2003) and Tonthubthimthong *et al*. (2001) that plant parts store plant compounds that can act as either repellents or pesticides. Therefore, the use of termite repellent plants in controlling termites is practical (Upadhyay *et al*., 2010; Prisila *et al*., 2014). However, the differences in effectiveness of the extractives from different plant parts may be as a result of differences in the plant compounds stored in different plant parts Gaya *et al*. (2013).

Phytochemicals Present in the Leaves of *S. Manii*

GC and HPLC analysis results of hexane and methanol extracts from the leaves of *S. manii* are presented in Fig. 5 and Fig. 6 respectively. GC indicates strong absorption at a wavelength of 240-290 nm typical of flavonoids and other phenolic compounds. Flavonoids and other phenolic compounds are usually present in plants in complex mixtures thus separated by a number of techniques such as GC or HPLC. It is important to note therefore that when flavonoids are seperated, different sub-classes such as flavonols, flavones, dihydroflavonols, flavan-3-ol, flavanones, anthyocyanidins, chalcones, dihydrochalcones and aurones can be obtained (Bhat, & Gupta, 2016; Abirami, & Rajendran, 2012; Nitu, Neelam, Bhavkiranbir, Supreet, & Gaganpreet, 2013; Proestos, Boziaris, Nychas, & Komaitis, 2006).

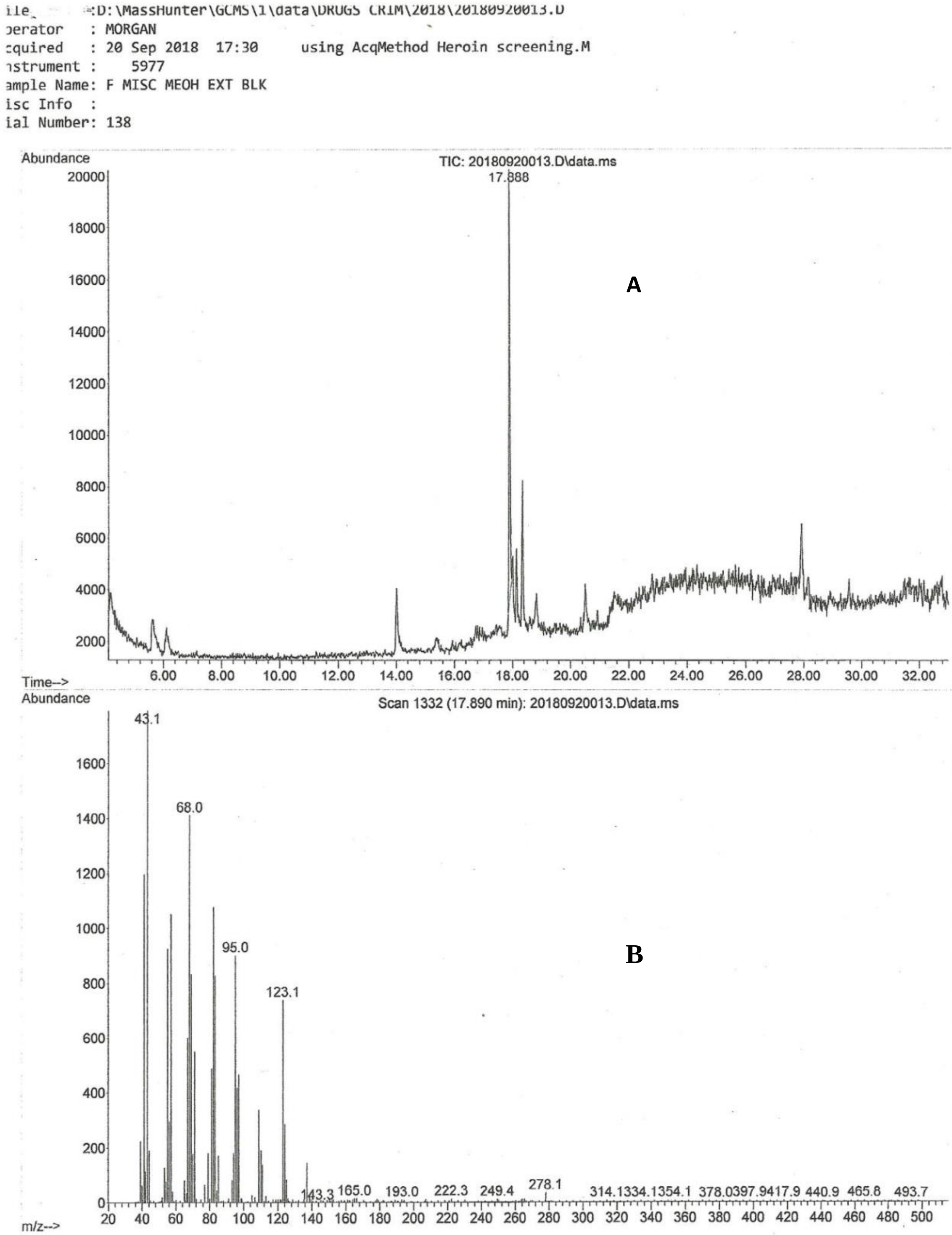


Figure 5: Gas chromatograph of reconstituted methanol extract from leaves (A) and spectrum of one of the components (B).

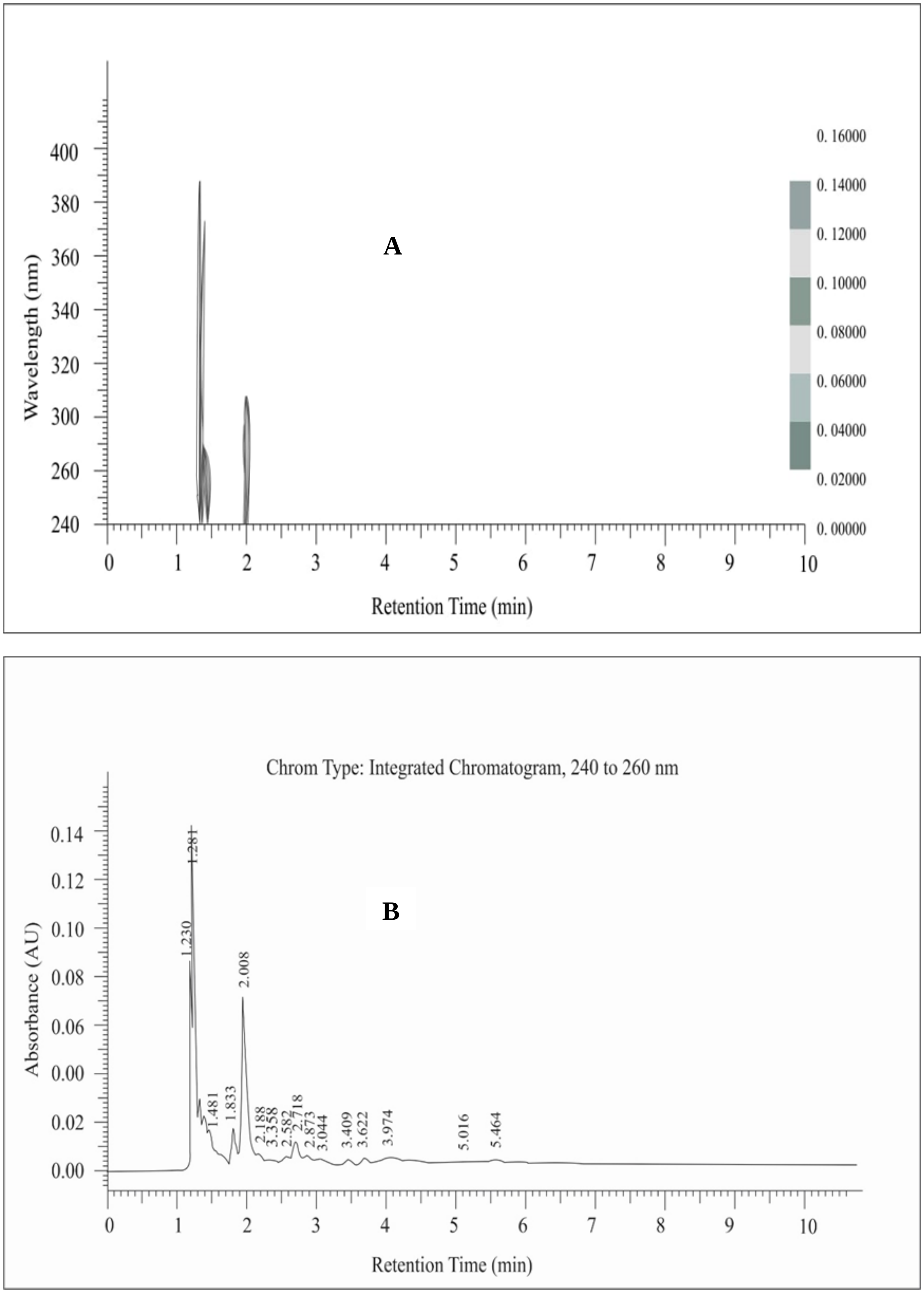


Figure 6: Gas chromatogam showing wavelengths of compounds (A) and compounds in the reconstituted methanol extract from leaves (B).

CONCLUSION

Ideed Nelisha (2007) observed that the concentration of essential oils, flavonoids, alkaloids and tannins is abundant in leaves and roots as compared to stems of plants however have different polarities. It is concluded that *S. manii* may have higher concentration of oils, flavanoids, alkaloids and tannins in its leaves that are responsible for its repellence ability to termites. Finally, the authors hereby make a declaration that this work did not have any conflict of interest in publication.

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