Composition of the Essential Oil and Insecticidal Activity of Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey Growing in Nigeria

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Abstract: The rice weevil (Sitophilus oryzae) is a pest of stored grain products such as rice, wheat, and corn. Essential oils represent a green environmentally-friendly alternative to synthetic pesticides for controlling stored-product insect pests. Launaea taraxacifolia is a leafy vegetable plant found in several parts of Nigeria. The leaves are eaten either fresh as a salad or cooked as a sauce. The essential oil obtained from fresh leaves of L. taraxacifolia was obtained by hydrodistillation and analyzed by gas chromatography/mass spectrometry (GC-MS). Twenty-nine compounds were identified, accounting for 100% of the oil composition. The major component classes were monoterpene hydrocarbons (78.1%), followed by oxygenated monoterpenoids (16.2%), sesquiterpene hydrocarbons (2.1%), oxygenated sesquiterpenoids (0.3%), and non-terpenoid derivatives (3.3%). The leaf essential oil was dominated by monoterpene hydrocarbons including limonene (48.8%), sabinene (18.8%), and (E)-β-ocimene (4.6%), along with the monoterpenoid aldehyde citronellal (11.0%). The contact insecticidal activity of L. taraxacifolia essential oil against Sitophilus oryzae was carried out; median lethal concentration (LC50) values of topical exposure of L. taraxacifolia essential oil were assessed over a 120-h period. The LC50 values ranged from 54.38 µL/mL (24 h) to 10.10 µL/mL (120 h). The insecticidal activity of the L. taraxacifolia essential oil can be attributed to major components limonene (48.8%), sabinene (18.8%), and citronellal (11.0%), as well as potential synergistic action of the essential oil components. This result showed L. taraxacifolia essential oil may be considered as a useful alternative to synthetic insecticides.

Keywords: essential oil composition; limonene; sabinene; citronellal; Sitophilus oryzae

1. Introduction

Insects such as Callosobruchus maculatus (Fabr.) (bruchid beetle), Sitophilus granarius (L.) (wheat weevil), S. oryzae (L.) (rice weevil), S. zeamais (Motsch.) (maize weevil), and Tribolium castaneum (Herbst) (red flour beetle), are important pests that attack stored grains, causing widespread economic losses [1–3]. The long-term use of synthetic insecticides to control these pests has become problematic, however. Compounds such as chlorinated hydrocarbons, organophosphates, carbamates, etc., tend to be toxic to non-target organisms such as mammals, birds, and fish [4–6], they are persistent in the environment [7–10], and many stored-grain insect pests have developed insecticide resistance [11–13]. Essential oils have emerged as viable alternatives to synthetic pesticides for control of stored-grain...
insect pests; they are generally non-toxic to mammals, birds, fish, or humans, have limited persistence, are readily biodegradable, and are renewable resources [14–17].

Launaea taraxacifolia (Willd.) Amin ex. C. Jeffrey (syn. Lactuca taraxacifolia (Willd.) Schumach, wild lettuce) is a leafy vegetable plant belonging to the Asteraceae (Compositae). The family consists of roughly 1100 genera, and 20,000 species distributed across several countries including Mexico, West Indies, Central and South America, Europe, North Africa, and tropical West African countries like Ghana, Senegal, Benin, and Nigeria [18]. L. taraxacifolia is a wild erect perennial herb that grows up to 1–3 m in height with 3–5 pinnately lobed leaves at the base of the stem in a rosette form. The plant is found singly or in clusters of rocky soil, but it is also cultivated in small open gardens near homes for family consumption. The leaves are eaten fresh as a salad or cooked as sauces [18–24]. The plant is known as ‘efo yanrin’ among the Yorubas of the southwestern part of Nigeria, ‘ugu’ among the Ibo of the eastern part of Nigeria, and ‘nonon barya’ among the Hausas of the northern part of Nigeria. Minerals, proteins, flavonoids, fatty acids, and vitamins have been reported to be found in the leaves of L. taraxacifolia [25,26]. The nutritional aspects of L. taraxacifolia have been reviewed [27,28]. The antioxidant and antiviral activities as well as the use of L. taraxacifolia leaves in treatment and control of blood cholesterol levels, blood pressure, and diabetes have been reported [29,30]. Phytochemical studies of L. taraxacifolia revealed that the plant possesses chemical classes such as phenolic glycosides, flavonoids, saponins and triterpenoids, which are known to have phytotherapeutic value for humans [25,31–34]. To the best of our knowledge, there is little or no information on the composition of the essential oil or the insecticidal activity of L. taraxacifolia. Therefore, the present research was undertaken with the aim of investigating the essential oil composition and evaluating the insecticidal potential of L. taraxacifolia leaves from southwestern Nigeria.

2. Materials and Methods

2.1. Plant Materials

The leaves of L. taraxacifolia were collected from Ipara, Badagry (6°4’54.07″ N and 2°52’52.75″ E) Lagos state, Nigeria. Botanical identification was done at the Herbarium, University of Lagos, Nigeria, where a voucher specimen (LUH: 7959) was deposited. Fresh leaves of L. taraxacifolia were cut into pieces, air dried, and pulverized in a blender to increase the surface area. A 450-g sample of blended L. taraxacifolia was hydrodistilled for 4 h in an all-glass modified Clevenger-type apparatus according to British Pharmacopoeia [35]. The obtained essential oil was stored in a sealed glass bottle with a screw lid cover under refrigeration at 4 °C until ready for use. Oil yield was calculated on a dry weight basis.

2.2. Gas Chromatographic–Mass Spectral Analysis

The chemical composition of L. taraxacifolia essential oil was determined by gas chromatography–mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, with a scan rate of 3.0 scans per s, with GC-MS solution software. The GC column was a ZB-5 fused silica capillary column (30 m length × 0.25 mm inner diameter) with a 5% phenyl-polydimethylsiloxane stationary phase and a film thickness of 0.25 μm. Helium gas was used as a carrier gas with column head pressure of 552 kPa at a flow rate of 1.37 mL/min. The injector temperature was 250 °C and the ion source temperature was 200 °C. The oven temperature of 50 °C was initially programmed for the GC and gradually increased at 2 °C/min to 260 °C. The sample (5% w/v) was dissolved in dichloromethane and 0.1 μL of the solution was injected using a split injection technique (30:1). Identification of the essential oil components was achieved by comparing the retention indices determined with respect to a homologous series of n-alkanes, and by comparison of the mass spectral fragmentation patterns with those stored in the MS databases [36–39].
2.3. Insecticidal Activity Screening

The essential oil was screened for insecticidal activity based on the method of Ilboudo and co-workers [40] with modifications. *Sitophilus oryzae* (L.) (rice weevil) were reared on whole rice (10:1 w/w). Adult insects, 1–7 days old, were used for contact toxicity tests. The insects were cultured in a dark growth chamber at a temperature of 27 ± 1 °C with relative humidity of 65 ± 5%. The insecticidal activity of *L. taraxacifolia* oil against *S. oryzae* (rice weevil) was evaluated by treatment of Whatman No. 1 filter paper discs with the essential oil diluted in ethanol. The required quantities of oil (0.10, 0.20, 0.30, and 0.40 µL) were diluted to 1 mL with ethanol and applied to filter paper discs, respectively. Permethrin (0.6% w/w) and ethanol were used as positive and negative controls, respectively. The solvent was allowed to evaporate from the filter paper, which was then placed into polyethylene cups (80 mm diameter). Ten well-fed mixed sex adult *S. oryzae* were introduced into the polyethylene cups, containing 20 g uninfected rice grains, and covered with a muslin cloth, held in place with rubber bands. Each treatment was replicated four times. Control experiments were set up as described as above without the essential oil. The experiment was arranged in a complete randomized design on a laboratory bench. The insect was considered dead when the legs or antennae were observed to be immobile. Insect mortalities were investigated by observing the recovery of immobilized insects after 24 h intervals for 120 h and the percentage of insect mortality was corrected using the Abbott formula [41]. Probit analysis [42] using XLSTAT version 2018.1.1.60987 (Addinsoft™, Paris, France) was used to estimate median lethal concentration (LC$_{50}$) values and insect toxicity data were analyzed using one-way ANOVA Tukey’s honestly significant difference test.

3. Results and Discussion

3.1. Essential Oil Composition

The essential oil from *L. taraxacifolia* was obtained by hydrodistillation with a yield of 1.68% as a pale-yellow essential oil, which was analyzed by GC-MS. The chemical composition of the leaf volatile oil of *L. taraxacifolia* is listed in Table 1. A total of 29 compounds were identified, accounting for 100% of the essential oil composition. The major chemical classes were monoterpene hydrocarbons (78%) and oxygenated monoterpenoids (16.2%), followed by sesquiterpene hydrocarbons (2.1%), oxygenated sesquiterpenoids (0.3%), and non-terpenoid derivatives (3.3%). The leaf essential oil was dominated by monoterpene hydrocarbons including limonene (48.8%), sabinene (18.8%), and (E)-β-ocimene (4.6%), along with the monoterpenoid aldehyde citronellal (11.0%). The chemical constituents of *L. taraxaciflora* essential oil have not been previously reported to the best of our knowledge. However, a phytochemical study and antioxidant and bacterial screening of the leaf extract of *L. taraxacifolia* have been reported [43].

| Constituents       | R$_{I_{calc}}$ | R$_{I_{db}}$ | Relative Abundance (%) |
|--------------------|---------------|--------------|------------------------|
| α-Pinene           | 941           | 933 [37]     | 0.9                    |
| Sabinene           | 976           | 971 [37]     | 18.8                   |
| Myrcene            | 993           | 991 [37]     | 2.2                    |
| α-Terpinene        | 1018          | 1018 [37]    | 0.6                    |
| Limonene           | 1032          | 1030 [37]    | 48.8                   |
| (Z)-β-ocimene      | 1042          | 1034 [37]    | 0.9                    |
| (E)-β-ocimene      | 1052          | 1045 [37]    | 4.6                    |
| γ-Terpinene        | 1062          | 1058 [37]    | 1.0                    |
| Terpinolene        | 1088          | 1086 [36]    | 0.4                    |
| Linalool           | 1101          | 1099 [38]    | 3.1                    |
| Citronellal        | 1155          | 1151 [38]    | 11.0                   |
| Terpinen-4-ol      | 1178          | 1180 [37]    | 1.4                    |
| 1-Dodecene         | 1192          | 1192 [39]    | 0.5                    |

Table 1. The chemical constituents of *Launaea taraxacifolia* leaf essential oil.
Table 1. Cont.

| Constituents          | RI_{calc} | RI_{db} | Relative Abundance (%) |
|-----------------------|-----------|---------|------------------------|
| n-Dodecane            | 1200      | 1200 [36] | 0.5                   |
| Neryl acetate         | 1366      | 1366 [39] | 0.7                   |
| 1-Tetradecene         | 1392      | 1388 [36] | 0.5                   |
| n-Tetradecane         | 1400      | 1400 [36] | 0.2                   |
| β-Caryophyllene       | 1420      | 1417 [36] | 1.5                   |
| α-Humulene            | 1456      | 1452 [36] | 0.1                   |
| Bicyclgermacrene      | 1495      | 1497 [38] | 0.3                   |
| Germacrene B          | 1556      | 1559 [36] | 0.2                   |
| Caryophyllene oxide   | 1581      | 1582 [36] | 0.3                   |
| 1-Hexadecene          | 1592      | 1588 [36] | 0.7                   |
| Pentadecanal          | 1712      | 1715 [38] | 1.0                   |

|                     |           |         |                       |
| Monoterpene hydrocarbons | 78.1   |         |                       |
| Oxygenated monoterpenoids | 16.2 |         |                       |
| Sesquiterpene hydrocarbons | 2.1   |         |                       |
| Oxygenated sesquiterpenoids | 0.3  |         |                       |
| Non-terpene derivatives | 3.3    |         |                       |
| Total identified (%)  | 100      |         |                       |

1 RI_{calc} = Kovats retention index determined with respect to a homologous series of n-alkanes on a ZB-5 column.
2 RI_{db} = Retention index from the databases [36–39].

3.2. Insecticidal Activity

The contact toxicity of *L. taraxacifolia* against *S. oryzae* revealed considerable differences in insect mortality rate to the essential oil with different concentrations and different exposure times. Table 2 shows that at a dose of 10.00 µL/mL, the volatile oil produced 25.00% mortality after 48 h (not significantly different than the negative EtOH control) and 52.50% after 120 h (significantly higher toxicity than the EtOH control). The essential oil produced 30.00%, 47.50%, 60.00%, and 75.00% mortality after 48, 72, 96, and 120 h at a dose of 20.00 µL/mL, respectively, while a dose of 30.00 µL/mL yielded a mortality rate of 42.50%, 57.50%, 75.00%, and 75.00%, respectively, over the same period of time. With longer contact times (≥48 h), 20 µL/mL and 30 µL/mL concentrations of *L. taraxacifolia* essential oil was significantly more toxic than the EtOH control, but less toxic than the permethrin positive control. The highest concentration of 40.00 µL/mL produced a mortality of 97.50%, and 100.00% after 96 and 120 h, respectively, which is significantly comparable to the permethrin positive control. Permethrin (0.6% w/w) against *S. oryzae* caused 40.0% mortality with 24 h of exposure and 100.0% mortality after 48 h. The negative control showed no appreciable activity against *S. oryzae* until after 120 h.

Table 2. Contact insecticidal effects of *Launaea taraxacifolia* essential oil on adult mortality of *Sitophilus oryzae* reared on rice grains 120 h after treatment.

| Mean % Mortality (±SE) | 24 h | 48 h | 72 h | 96 h | 120 h |
|------------------------|------|------|------|------|-------|
| Concentration (µL/mL)  |      |      |      |      |       |
| 10.00                  | 7.50 ± 5.00 c,d | 25.00 ± 12.91 c,d | 25.00 ± 12.91 d,e | 25.00 ± 12.91 c | 52.50 ± 17.08 c |
| 20.00                  | 15.00 ± 5.77 c,d | 30.00 ± 14.14 c | 47.50 ± 17.08 c,d,e | 60.00 ± 14.14 b,c | 75.00 ± 5.77 b |
| 30.00                  | 22.50 ± 9.57 b,c | 42.50 ± 12.58 h,c | 57.50 ± 9.57 b,c,e | 75.00 ± 5.77 b | 75.00 ± 5.77 b |
| 40.00                  | 45.00 ± 17.32 a | 65.00 ± 12.91 b | 75.00 ± 5.77 b | 97.50 ± 5.00 a | 100.00 ± 0.00 a |
| EtOH control           | 2.50 ± 5.00 d | 5.00 ± 5.77 d | 10.00 ± 8.16 e | 12.50 ± 9.57 c | 25.00 ± 5.77 d |
| Permethrin             | 40.00 ± 0.00 a,b | 100.00 ± 0.00 a | 100.00 ± 0.00 a | 100.00 ± 0.00 a | 100.00 ± 0.00 a |
| F-value, DF 2          | 15.08, 5 | 37.44, 5 | 39.69, 5 | 62.21, 5 | 51.13, 5 |

1 Mean followed by different letters in a column is significantly different at (p < 0.05). Insect toxicity data were analyzed using one-way ANOVA followed by Tukey’s test. 2 Degrees of freedom.
Median lethal concentration (LC$_{50}$) values at 95% confidence limits over exposure of _L. taraxacifolia_ essential oil were assessed and are shown in Table 3. After 120 h of exposure with an increase in concentration at regular intervals of 24 h, the LC$_{50}$ values were 54.38, 31.64, 21.48, 16.38, and 10.10 µL/mL, respectively. In this study, the essential oil of _L. taraxacifolia_ demonstrated contact toxicity to _S. oryzae_, since it had higher insecticidal activity with increasing essential oil concentration and exposure time. This result showed _L. taraxacifolia_ essential oil to have promising insecticidal activity against _S. oryzae_ and therefore may be considered as a useful, environmentally benign alternative to synthetic insecticides.

Table 3. Median lethal concentrations (LC$_{50}$, µL/mL, and 95% confidence limits) of _Larunaca taraxacifolia_ essential oil against _Sitophilus oryzae_.

| Contact Time | 24 h | 48 h | 72 h | 96 h | 120 h |
|--------------|------|------|------|------|-------|
| (95% confidence limits) | (39.26–133.8) | (23.86–55.67) | (16.62–27.21) | (13.56–18.78) | (5.67–13.31) |
| LC$_{50}$ | 54.38 | 31.64 | 21.48 | 16.38 | 10.10 |

To best of our knowledge, there have been no previous literature reports on the insecticidal activity of _L. taraxacifolia_ essential oil against _S. oryzae_ insect pest. However, contact toxicity of both limonene and sabinen tone, the major chemical components in this present study, have shown insecticidal activity against _S. oryzae_ [44]. Limonene has been previously reported to have a moderate contact effect against _S. zeamais_ (LD$_{50}$ values of 198.66 µg/cm$^2$) and _S. oryzae_ (with LD$_{50}$ of 260.18 µg/cm$^2$) [45] as well as fumigant toxicity against _S. oryzae_ (24-h LC$_{50}$ 61.5 µL/L) [46]. Garcia et al. reported that limonene showed contact toxicity against _T. castaneum_ [47]. Sabinenone, on the other hand, demonstrated weaker insecticidal activity against _S. oryzae_ (24-h LC$_{50}$ 463 µL/L) [44]. Interestingly, the _S. oryzae_ fumigant insecticidal activities of limonene and sabinene parallel the acetylcholinesterase (AChE) inhibitory activities; AChE IC$_{50} = 9.57$ µL/mL and 85.03 µL/mL, respectively, for limonene and sabinenone [48]. Furthermore, the binary combination of limonene + sabinenone showed synergistic AChE inhibition [48]. The insecticidal activity of the _L. taraxacifolia_ essential oil could be attributed to those known major components and the resulting synergistic action of the monoterpenic hydrocarbons limonene (48.8%) and sabinenone (18.8%).

The major aldehyde essential oil component, citronellal (11.0%), has also shown contact insecticidal activity against _Musca domestica_ [49] and _S. oryzae_ [50] and fumigant insecticidal activity against _T. castaneum_ [51] and _S. zeamais_ [52]. (−)-Citronellal has also shown AChE inhibitory activity with LC$_{50}$ of 18.4 mM [50]. The contact toxicities of 1,8-cineole, (+)-limonene, myrcene, α-phellandrene, α-pinene, sabinenone, and terpinolene, essential oil constituents obtained from leaves of _Chamaecyparis obtusa_, against _Callosobruchus chinensis_ (L.) and _Sitophilus oryzae_ (L.) have been reported [44]. The insecticidal activity of the essential oil components 1,8-cineole, p-cymene, α-pinene, and limonene has been previously reported with the order of activity 1,8-cineole > p-cymene > α-pinene > limonene [46]. Abdelgaleil et al. reported a comparative study of eleven monoterpenes contact and fumigant toxicity: camphene, (+)-camphor, (−)-carvone, 1,8-cineole, cuminaldehyde, (L)-fenchone, geraniol, (−)-limonene, (−)-linalool, (−)-menthol, and myrcene, against two important stored products insects, _S. oryzae_, and _T. castaneum_, and discovered that the toxicity varied according to insect pest with _S. oryzae_ more susceptible to most of the components than _T. castaneum_ [53].

4. Conclusions

This study investigated the essential oil composition and evaluated the insecticidal potential of _L. taraxacifolia_ leaves for the first time as a potential substitute to synthetic insecticides. _L. taraxacifolia_ offers an advantage in Nigeria due to its accessibility and renewability. Despite many advantages of medicinal plants, especially the essential oils, further studies need to be conducted to ascertain the safety of this essential oil before its practical use as an insecticide for controlling stored product insect pests. In addition, while the insecticidal properties of _L. taraxacifolia_ essential oil are promising,
this work is preliminary and future investigations extrapolating the use of the essential oil under grain-storage conditions should be pursued. In addition, studies on the controlled-release formulations of the essential oil could be examined to curb some of the challenges of essential oil treatments such as rapid degradation, volatility, and low bioavailability of the essential oils.

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