applied sciences

MDPI

Article

Essential Oil Compositions and Antifungal Activity of Sunflower (*Helianthus*) Species Growing in North Alabama

Sims K. Lawson [1,2], Layla G. Sharp [1,2], Chelsea N. Powers [2], Robert L. McFeeters [2], Prabodh Satyal [3] and William N. Setzer [2,3,*]

1 Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 35899, USA
2 Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
3 Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA
* Correspondence: wsetzer@chemistry.uah.edu; Tel.: +1-256-824-6519

Received: 1 May 2019; Accepted: 2 August 2019; Published: 5 August 2019

Abstract: *Helianthus* species are North American members of the Asteraceae, several of which have been used as traditional medicines by Native Americans. The aerial parts of two cultivars of *Helianthus annuus*, "Chianti" and "Mammoth", and wild-growing *H. strumosus*, were collected from locations in north Alabama. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography—mass spectrometry. The *Helianthus* essential oils were dominated by monoterpene hydrocarbons, in particular α-pinene (50.65%, 48.91%, and 58.65%, respectively), sabinene (6.81%, 17.01%, and 1.91%, respectively), β-pinene (5.79%, 3.27%, and 4.52%, respectively), and limonene (7.2%, 7.1%, and 3.8%, respectively). The essential oils were screened against three opportunistic pathogenic fungal species, *Aspergillus niger*, *Candida albicans*, and *Cryptococcus neoformans*. The most sensitive fungus was *C. neoformans* with minimum inhibitory concentration (MIC) values of 78, 156, and 78 μg/mL, respectively.

Keywords: *Helianthus annuus*; *Helianthus strumosus*; *Aspergillus niger*; *Candida albicans*; *Cryptococcus neoformans*; α-pinene

1. Introduction

Helianthus L., the sunflowers, is a genus in the family Asteraceae, tribe Heliantheae, made up of 51 North American species [1]. *Helianthus annuus* L. (common sunflower) is native to North America and the current range of wild forms of *H. annuus* are central and western United States, southern Canada, and northern Mexico [2]. The common sunflower is one of the earliest domesticated plants in the Americas. There is evidence that the plant was domesticated in Tabasco, Mexico, around 2600 B.C. [3], and independently in the southeastern United States around 2800 B.C. [2,4]. Several Native American tribes used *H. annuus* in traditional medicine [5]. For example, the White Mountain Apache used a poultice of the crushed plants to treat snakebites; the Hopi used the plant as a spider bite medicine; the Jemez applied the juice of the plant to cuts; the Pima used a decoction of the leaves to treat fevers [5]; and the Zuni natives of New Mexico used the roots to treat rattlesnake bites [6]. In addition, *H. annuus* is used as a traditional herbal medicine in many locations where it has been introduced. Ethiopians use *H. annuus* in teas to treat food poisoning [7]. In Bangladesh the seeds and/or the flowers are crushed and used for snake bites, scorpion bites, and a variety of other ailments, such as burning sensation in the vagina and worms in the ears [8].

Helianthus strumosus L. (woodland sunflower) is a rhizomatous perennial plant, growing up to two meters tall and is native to eastern North America [9–11]. These plants are strongly aromatic.

Leaves are up to 10 cm long and cuneate to subcordate in shape. The composite flower heads can be up to 9 cm at the peduncle. The ray flowers are a dark yellow color with orange-brown disc flowers in the center. These flowers are common along roadsides and in open fields and are sometimes found in forests. The Iroquois used a decoction of the roots as an anthelmintic [5].

Invasive fungal infections are becoming increasingly common in immunocompromised patients, such as those receiving cancer chemotherapy, transplant patients receiving immunosuppressant drugs, and HIV patients [12]. The predominant fungal pathogens are *Aspergillus* spp. [13,14] and *Candida* spp. [15,16] among others [12]. *Aspergillus niger* is a haploid filamentous parasitic fungus that is commonly known for the disease "black mold" on fruits, vegetables, and nuts [17]. *Aspergillus* conidia (fungal "spores") are environmentally widespread and inhalation can lead to opportunistic pulmonary aspergillosis, chiefly attributed to *A. fumigatus*, *A. flavus*, and *A. tubingensis*, as well as *A. niger* [18]. In immunocompromised individuals, however, the infection can progress to invasive systemic aspergillosis [19]. *Candida albicans* is another opportunistic pathogenic fungus that commonly colonizes the human body [20]. The organism can cause superficial infections of the mucosa, but can lead to invasive candidiasis in immunocompromised patients [21]. Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* [22]. The fungus is widespread in the environment and typically enters the body through inhalation where it can cause pulmonary infection [23]. However, the organism has the ability to cross the blood brain barrier and in immunocompromised patients, cryptococcosis can lead to cryptococcal meningoencephalitis with increased intracranial pressure [24,25]. As part of our continuing investigation of antifungal activity of essential oils [26] as well as essential oils from the Asteraceae growing in north Alabama [27], we have collected and analyzed the essential oils from the aerial parts of *H. annuus* and *H. strumosus*, and we have carried out in vitro antifungal screening of the essential oils against *A. niger*, *C. albicans*, and *C. neoformans*.

2. Materials and Methods

2.1. Plant Materials

The two cultivars of *H. annuus* ("Chianti" and "Mammoth") were cultivated, grown without fertilizer or pesticides, in a rural area near Gurley in north Alabama (34°38′29″N, 86°24′39″W, elevation 199 m) and the aerial parts were collected on 4 and 6 August 2018. Aerial parts of *H. strumosus* were collected on 10 August 2018 from wild-growing plants near Huntsville, Alabama (34°42′42″N, 86°32′35″W, elevation 354 m). The plants were identified by S.K. Lawson. Voucher specimens have been deposited in the herbarium of the University of Alabama in Huntsville (20180729-183243 and 20190402-111732). The fresh plant materials (78.14, 80.32, and 65.47 g, respectively) were hydrodistilled using a Likens–Nickerson apparatus with continuous extraction with dichloromethane for 3 h. The dichloromethane was carefully evaporated, and the residual essential oils weighed using an analytical balance to give the essential oils (82.3, 20.3, and 24.0 mg, respectively).

2.2. Gas Chromatographic—Mass Spectral Analysis

The *Helianthus* essential oils were analyzed by GC-MS with a Shimadzu GCMS-QP2010 Ultra with a ZB-5 capillary column as previously described [28]. Identification of the chemical components was carried out by comparison of the retention indices, calculated with respect to a homologous series of normal alkanes using the arithmetic index [29], and by comparison of their mass spectra with those reported in the Adams [30], NIST17 [31], FFNSC 3 [32], and our own in-house library [33]. Concentrations shown in Table 1 (average of three measurements ± standard deviations) are based on peak integration without standardization.

Table 1. Chemical compositions of *Helianthus annuus* "Chianti", *H. annuus* "Mammoth", and *H. strumosus* aerial parts essential oils.

RI [a]	RI [b]	Compound	Percent Composition [c]		
			***H. annuus* "Chianti"**	***H. annuus* "Mammoth"**	***H. strumosus***
800	797	(3*Z*)-Hexenal	0.06 ± 0.01	Tr [d]	tr
801	801	Hexanal	0.35 ± 0.02	0.24 ± 0.04	0.41 ± 0.03
810	796	2-Hexanol	—	—	0.07 ± 0.00
849	846	(2*E*)-Hexenal	1.13 ± 0.05	0.83 ± 0.05	1.96 ± 0.03
861	854	(2*E*)-Hexenol	—	—	tr
864	863	1-Hexanol	—	—	0.09 ± 0.00
921	921	Tricyclene	0.37 ± 0.00	0.21 ± 0.01	0.18 ± 0.00
924	924	α-Thujene	0.17 ± 0.00	0.23 ± 0.01	0.1
932	932	α-Pinene	50.65 ± 0.32	48.91 ± 0.64	58.65 ± 0.14
946	945	α-Fenchene	—	—	tr
948	946	Camphene	7.26 ± 0.03	3.72 ± 0.03	3.38 ± 0.02
952	953	Thuja-2,4(10)-diene	0.05 ± 0.01	—	tr
971	969	Sabinene	6.81 ± 0.04	17.01 ± 0.18	1.91 ± 0.00
977	974	β-Pinene	5.79 ± 0.04	3.27 ± 0.01	4.52 ± 0.02
988	988	Myrcene	0.42 ± 0.01	0.30 ± 0.03	9.79 ± 0.03
1004	1003	*p*-Mentha-1(7),8-diene	—	—	tr
1006	1002	α-Phellandrene	—	0.08 ± 0.01	0.05 ± 0.01
1008	1008	δ-3-Carene	—	—	tr
1016	1014	α-Terpinene	—	tr	—
1024	1020	*p*-Cymene	0.06 ± 0.03	0.09 ± 0.01	0.07 ± 0.00
1028	1024	Limonene	7.20 ± 0.03	7.11 ± 0.11	3.79 ± 0.01
1030	1025	β-Phellandrene	0.24 ± 0.00	0.21 ± 0.14	0.29 ± 0.01
1031	1026	1,8-Cineole	0.06 ± 0.00	0.07 ± 0.02	tr
1034	1032	(*Z*)-β-Ocimene	—	—	tr
1044	1044	(*E*)-β-Ocimene	—	tr	0.41 ± 0.01
1057	1054	γ-Terpinene	0.10 ± 0.00	0.25 ± 0.01	tr
1069	1065	*cis*-Sabinene hydrate	—	—	tr
1084	1086	Terpinolene	0.10 ± 0.01	0.16 ± 0.01	tr
1099	1099	α-Pinene oxide	0.10 ± 0.01	—	tr
1105	1100	Nonanal	—	—	tr
1109	1108	*p*-Mentha-2,8-dien-1-ol	0.36 ± 0.00	0.10 ± 0.02	tr
1112	1114	(3*E*)-4,8-Dimethyl-1,3,7-nonatriene	0.09 ± 0.01	0.13 ± 0.02	0.18 ± 0.00
1121	1124	Chrysanthenone	0.05 ± 0.00	—	—
1127	1122	α-Campholenal	0.33 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
1140	1135	*trans*-Pinocarveol	0.37 ± 0.05	tr	0.06 ± 0.02
1141	1137	*cis*-Verbenol	0.10 ± 0.01	—	tr
1145	1140	*trans*-Verbenol	1.50 ± 0.02	0.24 ± 0.02	0.26 ± 0.00
1163	1160	Pinocarvone	0.11 ± 0.01	tr	tr
1171	1165	Borneol	0.73 ± 0.01	0.07 ± 0.02	0.12 ± 0.00
1180	1174	Terpinen-4-ol	0.09 ± 0.01	0.19 ± 0.01	tr
1187	1179	*p*-Cymen-8-ol	0.05 ± 0.01	—	—
1195	1186	α-Terpineol	—	tr	—
1195	1195	Myrtenal	0.29 ± 0.02	tr	0.10 ± 0.01
1206	1201	Decanal	tr	—	—
1207	1204	Verbenone	0.28 ± 0.04	0.11 ± 0.01	0.07 ± 0.00
1219	1215	*trans*-Carveol	0.16 ± 0.00	—	tr
1283	1287	Bornyl acetate	7.13 ± 0.04	3.02 ± 0.04	4.97 ± 0.01
1294	1298	*trans*-Pinocarvyl acetate	0.08 ± 0.01	tr	tr
1382	1387	β-Bourbonene	0.21 ± 0.02	0.18 ± 0.01	tr
1386	1387	β-Cubebene	tr	tr	tr

Table 1. *Cont.*

RI [a]	RI [b]	Compound	Percent Composition [c]		
			H. annuus "Chianti"	*H. annuus* "Mammoth"	*H. strumosus*
1387	1389	β-Elemene	0.05 ± 0.01	0.17 ± 0.01	tr
1392	1392	(*Z*)-Jasmone	—	—	tr
1416	1419	β-Ylangene	0.07 ± 0.01	0.15 ± 0.01	tr
1417	1417	β-Caryophyllene	0.33 ± 0.03	0.54 ± 0.09	0.84 ± 0.00
1427	1434	γ-Elemene	—	—	tr
1428	1431	β-Gurjunene (=Calarene)	0.62 ± 0.01	0.86 ± 0.01	tr
1430	1432	*trans*-α-Bergamotene	0.06 ± 0.00	0.14 ± 0.03	tr
1442	1442	6,9-Guaiadiene	tr	tr	—
1446	1453	Geranyl acetone	tr	tr	—
1454	1452	α-Humulene	0.19 ± 0.02	0.29 ± 0.01	0.20 ± 0.00
1479	1484	Germacrene D	3.32 ± 0.03	6.84 ± 0.09	3.68 ± 0.02
1487	1489	β-Selinene	0.12 ± 0.01	tr	tr
1493	1493	*epi*-Cubebol	0.12 ± 0.04	—	—
1494	1500	Bicyclogermacrene	—	0.16 ± 0.01	0.07 ± 0.01
1513	1514	Cubebol	0.14 ± 0.02	tr	tr
1516	1522	δ-Cadinene	tr	0.07 ± 0.00	tr
1547	1548	Elemol	—	tr	0.07 ± 0.01
1559	1561	(*E*)-Nerolidol	0.10 ± 0.02	0.09 ± 0.03	0.64 ± 0.03
1575	1574	Germacrene D-4β-ol	0.37 ± 0.02	0.46 ± 0.00	0.46 ± 0.00
1581	1582	Caryophyllene oxide	0.16 ± 0.09	tr	0.37 ± 0.01
1608	1608	Humulene epoxide II	—	—	0.05 ± 0.01
1636	1639	Caryophylla-4(12),8(13)-dien-5β-ol	—	—	tr
1638	1643	Hedycaryol	—	—	0.10 ± 0.01
1641	1638	τ-Cadinol	0.18±0.01	tr	0.14 ± 0.01
1654	1649	β-Eudesmol	0.10 ± 0.02	—	0.16 ± 0.03
1655	1652	α-Cadinol	—	tr	tr
1663	1665	Intermediol	tr	0.56 ± 0.01	—
1683	1685	Germacra-4(15),5,10(14)-trien-1α-ol	0.13 ± 0.04	—	0.51 ± 0.01
1686	1687	Eudesma-4(15),7-dien-1β-ol	—	—	0.14 ± 0.00
1689	1690	(Z)-*trans*-α-Bergamotol	—	—	0.12 ± 0.01
1699	1695	6-*epi*-Shyobunol	—	—	0.05 ± 0.01
		Monoterpene hydrocarbons	79.21	81.56	83.13
		Oxygenated monoterpenoids	11.80	3.85	5.64
		Sesquiterpene hydrocarbons [e]	4.97	9.40	4.79
		Oxygenated sesquiterpenoids [e]	1.39	1.11	2.79
		Green leaf volatiles	1.54	1.07	2.53
		Others	0.091	0.13	0.18
		Total Identified	99.01	97.12	99.05

[a] RI = Retention index determined with reference to a homologous series of *n*-alkanes on a ZB-5 column. [b] RI values from the databases (NIST17 [31], FFNSC 3 [32], Adams [30], or Satyal [33]). [c] Average of three measurements ± standard deviations. [d] tr = "trace" (<0.05%). [e] Sesquiterpenoids are considered tentatively identified based on MS and RI.

2.3. Antifungal Screening Assays

The *Helianthus* essential oils were screened for antifungal activity against *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 18804), and *Cryptococcus neoformans* (ATCC 24607) using the broth dilution technique as previously described [26,34]. Antifungal screening was carried out in triplicate.

3. Results and Discussion

3.1. Essential Oil Compositions

Hydrodistillation of *Helianthus* aerial parts gave pale yellow essential oils in 0.105%, 0.025%, and 0.037% yield (*w*/*w*) for *H. annuus* "Chianti", *H. annuus* "Mammoth", and *H. strumosus*, respectively.

The essential oil compositions for the three essential oils are compiled in Table 1. A perusal of the table reveals that the three *Helianthus* essential oils are qualitatively similar. The major components for *H. annuus* "Chianti" were α-pinene (50.65%), camphene (7.26%), limonene (7.20%), bornyl acetate (7.13%), sabinene (6.81%), and β-pinene (5.79%). The essential oil of *H. annuus* "Mammoth" was also dominated by α-pinene (48.91%), followed by sabinene (17.01%), limonene (7.11%), and germacrene D (6.84%). *H. strumosus* essential oil was also rich in α-pinene (58.65%), as well as myrcene (9.79%) and bornyl acetate (4.97%).

The compositions of *H. annuus* essential oils cultivated in north Alabama are very similar to those reported by Adams and co-workers for populations growing in the southern plains of the United States [35]. The essential oils of *H. annuus* from Pisa, Tuscany, Italy [36]; Lagos, Nigeria [37]; or from western United States [35] had much lower concentrations of α-pinene and correspondingly higher concentrations of germacrene D. In marked contrast to the essential oils of *Helianthus*, essential oils of *Rudbeckia fulgida* Aiton and *Rudbeckia hirta* L. (Asteraceae, Heliantheae) from north Alabama were devoid of α-pinene, but rich in sesquiterpene hydrocarbons [27].

3.2. Antifungal Activity

The *Helianthus* essential oils were screened for antifungal activity against three potentially pathogenic fungi, *Aspergillus niger*, *Candida albicans*, and *Cryptococcus neoformans*, as shown in Table 2. The most susceptible fungus was *C. neoformans*. Both *H. annuus* "Chianti" and *H. strumosus* essential oils showed minimum inhibitory concentration (MIC) values of 78 μg/mL. It is tempting to suggest that the major component, α-pinene, is responsible for the observed anti-*Cryptococcus* activity; all three *Helianthus* essential oils have around 50% α-pinene. Furthermore, α-pinene has shown antifungal activity against *C. neoformans* with an MIC around 70 μg/mL [38,39]. In addition, α-pinene-rich (46.1% α-pinene) commercial *Myrtis communis* essential oil showed a similar antifungal activity against *C. neoformans* (MIC = 78 μg/mL) [26]. Conversely, commercial *Cupressus sempervirens* essential oil, with 49.7% α-pinene was less active against *C. neoformans* (MIC = 313 μg/mL) [26]. There may be synergistic or antagonistic effects of α-pinene with minor components. Limonene [39,40] and β-pinene [39], have also shown antifungal activity against *C. neoformans*; camphene, however, was inactive [41]. Although we do not know which of the enantiomers is present in the *Helianthus* essential oils, we have screened both (+)- and (−)-α-pinene, (+)- and (−)-limonene, and (−)-β-pinene against the three fungal strains, as shown in Table 2. Consistent with previous investigations, (−)-β-pinene and (+)-limonene both showed activity against *C. neoformans* with MIC values of 39 and 78 μg/mL. Furthermore, both enantiomers of α-pinene were active against *C. neoformans*; MIC = 20 and 39 μg/mL for (+)- and (−)-α-pinene, respectively.

Table 2. Antifungal activities (minimum inhibitory concentration (MIC), μg/mL) of *Helianthus* essential oils and major components [a].

Material	Fungal Species		
	Aspergillus niger	***Candida albicans***	***Cryptococcus neoformans***
H. annuus "Chianti"	625	625	78
H. annuus "Mammoth"	625	625	156
H. strumosus	625	1250	78
(+)-α-Pinene	625	313	20
(−)-α-Pinene	156	625	39
(−)-β-Pinene	156	625	39
(+)-Limonene	1250	625	78
(−)-Limonene	2500	1250	313
Amphotericin B	0.78	0.78	1.56

[a] Each MIC determination was carried out in triplicate.

4. Conclusions

Helianthus essential oils have been shown to be rich in α- and β-pinenes, sabinene, and limonene, and have demonstrated poor antifungal activities against *A. niger* and *C. albicans*, but promising activity against *C. neoformans* (although much lower activity than the reference antifungal drug amphotericin B). These and other monoterpene-rich essential oils deserve further exploration as alternative and complementary agents to combat fungal infections; further studies against more susceptible fungi are recommended.

Author Contributions: Conceptualization, S.K.L. and W.N.S.; methodology, S.K.L., L.G.S. and C.N.P.; software, P.S.; validation, W.N.S.; formal analysis, W.N.S.; investigation, S.K.L., L.G.S. and C.N.P.; resources, R.L.M.; data curation, W.N.S.; writing—original draft preparation, S.K.L., L.G.S. and W.N.S.; writing—review and editing, all authors; project administration, W.N.S.

Funding: This research received no external funding.

Acknowledgments: P.S. and W.N.S. participated in the project as part of the activities of the Aromatic Plant Research Center (APRC, https://aromaticplant.org/).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mabberley, D.J. *Mabberley's Plant-Book*, 3rd ed.; Cambridge University Press: Cambridge, UK, 2008.
2. Smith, B.D. Eastern North America as an independent center of plant domestication. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12223–12228. [CrossRef] [PubMed]
3. Lentz, D.L.; Pohl, M.D.; Alvarado, J.L.; Tarighat, S.; Bye, R. Sunflower (*Helianthus annuus* L.) as a pre-Columbian domesticate in Mexico. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6232–6237. [CrossRef] [PubMed]
4. Blackman, B.K.; Scascitelli, M.; Kane, N.C.; Luton, H.H.; Rasmussen, D.A.; Bye, R.A.; Lentz, D.L.; Rieseberg, L.H. Sunflower domestication alleles support single domestication center in eastern North America. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14360–14365. [CrossRef] [PubMed]
5. Moerman, D.E. *Native American Ethnobotany*; Timber Press, Inc.: Portland, OR, USA, 1998.
6. Camazine, S.; Bye, R.A. A study of the medical ethnobotany of the Zuni Indians of New Mexico. *J. Ethnopharmacol.* **1980**, *2*, 365–388. [CrossRef]
7. Mesfin, F.; Demissew, S.; Teklehaymanot, T. An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J. Ethnobiol. Ethnomed.* **2009**, *5*, 28. [CrossRef] [PubMed]
8. Rahman, A.H.M.M. Medico-ethnobotany: A study on the tribal people of Rajshahi division, Bangladesh. *Peak J. Med. Plant Res.* **2016**, *1*, 1–8.
9. Kartesz, J.T. BONAP's North American Plant Atlas. Available online: http://bonap.net/Napa/TaxonMaps/Genus/County/Helianthus (accessed on 19 April 2019).
10. Tropicos.org. Missouri Botanical Garden. Available online: www.tropicos.org (accessed on 19 April 2019).
11. eFloras.org. *Helianthus Strumosus* Linnaeus. Available online: http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242416641 (accessed on 24 April 2019).
12. Richardson, M.; Lass-Flörl, C. Changing epidemiology of systemic fungal infections. *Clin. Microbiol. Infect.* **2008**, *14*, 5–24. [CrossRef] [PubMed]
13. Erjavec, Z.; Kluin-Nelemans, H.; Verweij, P.E. Trends in invasive fungal infections, with emphasis on invasive aspergillosis. *Clin. Microbiol. Infect.* **2009**, *15*, 625–633. [CrossRef]
14. Galimberti, R.; Torre, A.C.; Baztán, M.C.; Rodriguez-Chiappetta, F. Emerging systemic fungal infections. *Clin. Dermatol.* **2014**, *30*, 633–650. [CrossRef]
15. Dean, D.A.; Burchard, K.W. Fungal infection in surgical patients. *Am. J. Surg.* **1996**, *171*, 374–382. [CrossRef]
16. Miceli, M.H.; Díaz, J.A.; Lee, S.A. Emerging opportunistic yeast infections. *Lancet Infect. Dis.* **2011**, *11*, 142–151. [CrossRef]
17. Hocking, A.D. *Aspergillus* and related teleomorphs. In *Food Spoilange Microorganisms*; Blackburn, C.d.W., Ed.; CRC Press: Boca Raton, FL, USA, 2006; pp. 451–487. ISBN 0-8493-9156-3.

18. Shittu, O.B.; Adelaja, O.M.; Obuotor, T.M.; Sam-Wobo, S.O.; Adenaike, A.S. PCR-internal transcribed spacer (ITS) genes sequencing and phylogenetic analysis of clinical and environmental *Aspergillus* species associated with HIV-TB co infected patients in a hospital in Abeokuta, southwestern Nigeria. *Afr. Health Sci.* **2016**, *16*, 141–148. [CrossRef] [PubMed]
19. Paulussen, C.; Hallsworth, J.E.; Álvarez-Pérez, S.; Nierman, W.C.; Hamill, P.G.; Blain, D.; Rediers, H.; Lievens, B. Ecology of aspergillosis: Insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microb. Biotechnol.* **2017**, *10*, 296–322. [CrossRef] [PubMed]
20. Kabir, M.A.; Hussain, M.A.; Ahmad, Z. *Candida albicans*: A model organism for studying fungal pathogens. *Int. Sch. Res. Netw. Microbiol.* **2012**, *2012*, 538694. [CrossRef] [PubMed]
21. Gow, N.A.R.; Yadav, B. Microbe profile: *Candida albicans*: A shape-changing, opportunistic pathogenic fungus of humans. *Microbiology* **2017**, *163*, 1145–1147. [CrossRef]
22. Hay, R.J. Fungal infections. *Clin. Dermatol.* **2006**, *24*, 201–212. [CrossRef]
23. Paterson, D.L.; Singh, N. *Cryptococcus neoformans* infection. *Liver Transpl.* **2002**, *8*, 846–847. [CrossRef]
24. Gaona-Flores, V.A. Central nervous system and *Cryptococcus neoformans*. *N. Am. J. Med. Sci.* **2013**, *5*, 492–493. [CrossRef]
25. Armstrong-James, D.; Meintjes, G.; Brown, G.D. A neglected epidemic: Fungal infections in HIV/AIDS. *Trends Microbiol.* **2014**, *22*, 120–127. [CrossRef]
26. Powers, C.N.; Osier, J.L.; McFeeters, R.L.; Brazell, C.B.; Olsen, E.L.; Moriarity, D.M.; Satyal, P.; Setzer, W.N. Antifungal and cytotoxic activities of sixty commercially-available essential oils. *Molecules* **2018**, *23*, 1549. [CrossRef]
27. Stewart, C.D.; Jones, C.D.; Setzer, W.N. Leaf essential oil compositions of *Rudbeckia fulgida* Aiton, *Rudbeckia hirta* L., and *Symphyotrichum novae-angliae* (L.) G.L. Nesom (Asteraceae). *Am. J. Essent. Oils Nat. Prod.* **2014**, *2*, 36–38.
28. Satyal, P.; Hieu, H.V.; Chuong, N.T.H.; Hung, N.H.; Sinh, L.H.; Van The, P.; Tai, T.A.; Hien, V.T.; Setzer, W.N. Chemical composition, *Aedes* mosquito larvicidal activity, and repellent activity against *Triatoma rubrofasciata* of *Severinia monophylla* leaf essential oil. *Parasitol. Res.* **2019**, *118*, 733–742. [CrossRef] [PubMed]
29. Van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471. [CrossRef]
30. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing: Carol Stream, IL, USA, 2007.
31. *NIST17*; National Institute of Standards and Technology: Gaithersburg, MD, USA, 2017.
32. Mondello, L. *FFNSC 3*; Shimadzu Scientific Instruments: Columbia, MD, USA, 2016.
33. Satyal, P. Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active Novel Chemotypes in Essential Oils. Ph.D. Thesis, University of Alabama in Huntsville, Huntsville, AL, USA, 2015.
34. Sahm, D.H.; Washington, J.A. Antibacterial susceptibility tests: Dilution methods. In *Manual of Clinical Microbiology*; Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg, H.D., Shamody, H.J., Eds.; American Society for Microbiology: Washington, DC, USA, 1991.
35. Adams, R.P.; TeBeest, A.K.; Holmes, W.; Bartel, J.A.; Corbet, M.; Parker, C.; Thornburg, D. Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). *Phytologia* **2017**, *99*, 130–138.
36. Ceccarini, L.; Macchia, M.; Flamini, G.; Cioni, P.L.; Caponi, C.; Morelli, I. Essential oil composition of *Helianthus annuus* L. leaves and heads of two cultivated hybrids "Carlos" and "Florom 350". *Ind. Crops Prod.* **2004**, *19*, 13–17.
37. Ogunwande, I.A.; Flamini, G.; Cioni, P.L.; Omikorede, O.; Azeez, R.A.; Ayodele, A.A.; Kamil, Y.O. Aromatic plants growing in Nigeria: Essential oil constituents of *Cassia alata* (Linn.) Roxb. and *Helianthus annuus* L. *Rec. Nat. Prod.* **2010**, *4*, 211–217.
38. Lima, I.O.; Oliveira, R.d.A.G.; Lima, E.d.O.; de Souza, E.L.; Farias, N.P.; Navarro, D.d.F. Inhibitory effect of some phytochemicals in the growth of yeasts potentially causing opportunistic infections. *Rev. Bras. Ciênc. Farm.* **2006**, *41*, 199–203. [CrossRef]
39. Pinto, E.; Hrimpeng, K.; Lopes, G.; Vaz, S.; Gonçalves, M.J.; Cavaleiro, C.; Salgueiro, L. Antifungal activity of *Ferulago capillaris* essential oil against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *Eur. J. Clin. Microbiol. Infect. Dis.* **2013**, *32*, 1311–1320. [CrossRef]

40. Pinto, E.; Gonçalves, M.J.; Cavaleiro, C.; Salgueiro, L. Antifungal activity of *Thapsia villosa* essential oil against *Candida*, *Cryptococcus*, *Malassezia*, *Aspergillus* and dermatophyte species. *Molecules* **2017**, *22*, 1595. [CrossRef]
41. Tirillini, B.; Velasquez, E.R.; Pellegrino, R. Chemical composition and antimicrobial activity of essential oil of *Piper angustifolium*. *Planta Med.* **1996**, *62*, 372–373. [CrossRef]