Comparative Study of Chemical Composition of the Essential Oils of *Turnera diffusa* (Josef August Schultes) Leaf and Stem

Isaac S. Njoku[1,2]; Misturah Olaleye[1]; and Olayinka T. Asekun[1]

[1]Department of Chemistry, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.
[2]Department of Chemistry, Faculty of Basic Medical and Applied Sciences, Trinity University, Lagos, Nigeria.

E-mail: isaac.njoku@trinityuniversity.edu.ng

ABSTRACT

The essential oils of *Turnera diffusa* (commonly called Damiana) leaves and stem were extracted by hydrodistillation and analyzed using Gas Chromatograph-Mass Spectrometry (GC-MS). *Turnera diffusa* leaf and stem yielded the same percentage of essential oils (0.6%), respectively. The major components of the leaf oil are α – pinene (15.47%), cubebol (9.59%), junenol (6.31%), spathunelol (6.12%), and caryophyllene (5.47%) while the major components of the stem oil are junenol (8.29%), germacrene D (7.42%), caryophyllene (4.21%), σ – cadinene (3.77%), and spathunelol (3.58%). The result showed that there was a considerable variation in the chemical composition of the leaf and stem essential oils of *T. diffusa*.

(Keywords: *Turnera diffusa*, damiana, essential oils, spathunelol, junenol, caryophyllene)

INTRODUCTION

Turnera diffusa, also known as Damiana, is a low-growing flowering plant with fragrant leaves and yellow blooms. It's native to southern Texas, Mexico, Central and South America, and the Caribbean, where it thrives in subtropical climes[1]. It is a member of the *Passifloraceae* family. The main bioactive components of *Turnera diffusa* include the fatty acids, alkaloids, phenolics (flavonoids, phenolic acids, and its derivatives), cyanogenic glycosides, sugar conjugate, and its essential oils which are obtained mainly from its leaves and stems[2]. Its leaves are used to enhance the flavor of dishes and liquors, and its infusions are utilized for a variety of therapeutic purposes, including as nervous system stimulants, aphrodisiacs, and diuretics[3].

The leaves contain up to 1% essential oil, which is made up of at least 20 bioactive components such as 1,8-cineole and -pinene, p-cymene, thymol, calamene, alpha-copaene, tannins, flavonoids, damianin, beta-sitosterol, arbutin, glycosides, gonzalitosin, and tetraphyllin B, among others[4]. According to recent studies, it exhibits tyrosinase inhibitory qualities[5] as well as testicular protection, pro-sexual effects in rats[6], sexual behaviour recovery[7], anti-aromatase activity[8], and gastroprotective properties[9].

The methanolic extract of *Turnera diffusa* showed that it has a cytotoxic effect on MDA-MB-231 breast cancer cells, and that this activity is due in part to apigenin activity[10]. The findings showed that *Turnera diffusa* has another therapeutic function, this time as a potential cancer alternative therapy.

Arbutin, a major constituent of Turnera *diffusa* evaluated in aspirin or ethanol-induced ulcer in vivo showed anti-ulcer activity, which could be attributed to lipid peroxidation inhibitory, immuno-modulatory and anti-oxidant mechanisms of arbutin[11].The research work by[12] is the first to show that the methanolic extract of *Turnera diffusa* has a cytotoxic effect on MDA-MB-231 breast cancer cells, and that this activity is due in part to apigenin activity. *Turnera diffusa* possesses anti-ulcer activity, which could be attributed to lipid peroxidation inhibitory, immunomodulatory, and antioxidant mechanisms of arbutin[13].

Damiana leaves have been used in traditional medicine as an aphrodisiac[14], diuretic, nerve tonic, urinary tract infections, frigidity, vaginal discharge, painful menstruation, and menopause problems[15]. The current importance of *damiana* in industrialized countries is not only as a medicinal plant but also as a drug for the treatment of sexual impotence where it is used in conjunction with other stimulants[16].

Though there are reports on the chemical composition of the essential oil of *T. diffusa*, there is very little research on the comparative analysis of the volatile components from different parts of this plant. It is on this premise that this research seeks to study the variations in chemical composition of the steam and leaf essential oil of *T. diffusa*.

MATERIALS AND METHODS

Plant Material and Essential Oil Extraction Technique

The fresh leaves and stems of *Turnera diffusa* were collected at Lekki, Lagos state, Nigeria in February, 2020. The plant was taxonomically identified and authenticated at the Herbarium of the Department of Botany, University of Lagos, Nigeria with the voucher number LUH: 8647. Prior to extraction, the plant was washed with running water, air-dried, and separated into stems and leaves, and then pulverised into coarse powder.

About 300g of the pulverized plant leaves and stems were separately subjected to hydro-distillation for 3 hours, using the modified Clevenger-type apparatus[17]. The extracted oil was dried over anhydrous sodium sulphate and concentrated by air-drying. The concentrated extracted oils were collected in air-tight vials and refrigerated prior to analysis using the GC-MS.

GC–FID and GC/MS Analyses of Volatile Oils

GC–FID and GC/MS Analyses of Volatile Oils The essential oil samples were analyzed using a Varian CP-3800 gas chromatograph fitted with a flame ionization detector (FID) and dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50 m length × 0.25 mm i.d. × 0.4 μm film thickness) (Varian, Netherlands). Nitrogen was the carrier gas with a 16-psi inlet pressure.

Samples (0.2 μL) were injected in split mode with a ratio of 1:100. The column was initially held at 60°C for 5 minutes then heated to 220°C at a 5°C/minute ramp rate and was held for 3 minutes at that temperature. The temperature was further raised to 250°C at a 5°C/minute ramp rate and was held at this temperature for 4 minutes. The injector and detector temperatures were maintained at 250° and 300°C, respectively.

The gas chromatography/mass spectrometry (GC/MS) analyses performed on a Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with a mass range of 40–500 amu, employing an Elite-5 column (5 % phenyl and 95 % dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness (PerkinElmer, USA). Helium (1 mL/min) was used as a carrier gas. The initial temperature was 60 °C (1 min), this was increased to 240 °C at rate of 6 °C/min, and remained at 240 °C for 6 min, and then continued to increase to 250 °C at rate of 10 °C/min, with a final stage of 10 min at 250 °C. The oven temperature was programmed from 50 °C to 250 °C at a 5 °C/min dynamic rate and remained for 15 min at 250 °C.

Samples (0.1 uL) were injected with a split less mode. Identification of Volatile Oil Constituents Component identification was accomplished by comparison of the retention indices (RI) of the GC peaks with those obtained using saturated n-alkanes (C8–C30) (Aldrich, USA), those reported in the literature[18,19,20,21] and by comparison of the mass spectra of the peaks with those reported in the literature (20,21) and stored in the NIST library. Peak area percentages were calculated from GC–FID response without employing correction factors. RI values were calculated for all components using a homologous series of n-alkane. Mixtures (C7-C30) injected under conditions similar to those of the samples and computer matched with the NIST libraries.

RESULTS AND DISCUSSION

The essential oil of *Turnera diffusa* leaf and stem were extracted using the hydro-distillation technique and analyzed using the GC – MS. A total of 28 components were identified in the total oil extract of *T. diffusa* leaf, while 44 components were identified for the total oil extract of its stem as shown in Table 1.

The oil yield was 0.6 % for both the stem and leaf essential oil. The oils from both the steam and leaves of *T. diffusa* were made up of predominantly mono and sesquiterpenes (Table 2). The major components the leaf essential oil of *T. diffusa* were α-pinene (15.47 %), cubebol (9.59 %), junenol (6.31 %), spathulenol (6.12 %), and caryophyllene (5.47 %).

Table 1: Comparison of the Composition of Chemical Components of the Essential Oils Obtained from *T. diffusa* Leaf and Stem.

Compound	Leaf %	Stem %	Rcal	RI	Standard Deviation
α-Pinene	15.47	0.28	931	933	10.74
β-Pinene	-	3.48	941	943	-
α-Pinene, (D)	-	0.28	944	948	-
α-phellandrene	-	1.13	967	969	-
D-Limonene	-	2.17	1020	1018	-
β-Cymene	-	1.39	1039	1042	-
Bicyclo[2.2.1]heptane,2-cyclopropylidene-1,7,7-trimethyl	-	0.72	1102	1106	-
α-Phellandrene-8-ol	-	1.95	1128	1125	-
L-Pinocarveol	1.29	1.47	1127	1131	0.13
Trans-Verbenol	3.85	-	1138	1136	-
Myrtenal	-	0.71	1139	1136	-
Terpinen-4-ol	-	1.21	1136	1137	-
Isoborneol	1.21	-	1140	1138	-
Terpineol	-	0.82	1146	1143	-
α-campholenal	-	2.10	1156	1154	-
Cis-Carveol	0.96	1.29	1203	1206	0.79
β-Copaene	3.21	0.72	1219	1216	1.76
Copaene	0.96	3.47	1222	1221	1.77
1H-Indene,1 ethylideneoctahydro-7a-methyl-(1Z,3aα,7aβ)	-	1.64	1236	1239	-
Eugenol	2.44	-	1391	1391	-
Silphiperfol-5-ene	3.21	2.99	1404	1403	0.16
Calarene	2.23	1.50	1400	1403	0.52
Aristolene	-	0.67	1400	1403	-
Isoledene	-	0.70	1421	1419	-
Elixene	-	1.08	1435	1431	-
Bicyclosesquiphellandrene	1.25	0.93	1437	1435	0.23
γ-Cadinene	-	2.80	1436	1435	-
α-Cubebene	0.95	0.60	1444	1440	0.25
σ-Cadinene	3.79	3.77	1438	1440	0.26
Cadina-3,5-diene	-	0.60	1442	1440	-
α-Selinene	2.41	-	1472	1474	-
Valencene	-	2.51	1476	1474	-
Cubebol	9.59	0.81	1486	1484	6.20
Caryophyllene	5.47	4.21	1496	1494	0.89
Caryophyllene oxide	4.80	1.69	1505	1507	2.20
Germacrene D	3.80	7.42	1517	1515	2.56
Cadina-1,4-diene	-	0.61	1521	1525	-
Spathulenol	6.12	3.58	1539	1536	1.79
Shyobunol	0.82	0.62	1552	1555	0.14
Nerolidol	0.87	1.04	1566	1564	0.12
Humulene	1.79	1.96	1581	1579	0.12
α-cardinol	3.96	2.56	1582	1580	0.98
Tetradecanal	-	1.02	1604	1601	-
Junenol	6.31	8.29	1622	1625	1.40
(1R,7S, E)-7-Isopropyl-4,10dimethylenecyclodec-5-enol	1.55	1.61	1702	1699	0.04
Hexahydrofarnesyl Acetone	1.06	0.63	1752	1754	0.30
Kaura-16-ene	-	0.55	1784	1789	-
Phytol	1.58	0.75	2041	2045	0.59
Cembrene A	0.71	-	2076	2072	-

Notes: RIcal, retention index calculated on CP Sil-5 column relative to C8–C20 n-alkanes; RI, retention index reported in the literature.

Table 2: Grouped Composition of Essential oil of *T. diffusa* Leaf and Stem.

Group	Leaf (%)	Steam (%)	Standard Deviation
Monoterpene	5.71	8.77	2.16
Monoterpenoid	17.14	17.54	0.28
Sesquiterpene	34.29	35.09	0.57
Sesquiterpenoid	25.71	15.79	7.01
Diterpene	2.86	5.26	2.40
Diterpenoid	11.43	8.77	1.88
Others	2.86	8.77	4.18

However, junenol (8.29%), germacrene D (7.42%), caryophyllene (4.21%) were the most prominent components in the total oil extract of *T. diffusa* stem. The variation in the chemical composition of the leaf and stem oil of *T. Diffusa* well marked as the stem oil had about Twenty components that were absent in the leaf essential oil, these include; tran verbenol, isoborneol, eugenol and α-selinene. The leaf essential oil also had five components not present in the stem essential oil, these include d-limonene, α-campholenal, γ-cadinene, and valencene.

Twenty-three components were however present in the total oil extract of *T. diffusa* leaf and stem, these include; junenol, α-pinene, σ-cadinene, caryophyllene, germacrene D and spathulenol. The oils also varied in their percentages of grouped components. The leave essential oil had higher composition of sesqui-and diterpenes while the stem had higher monoterpene composition.

Earlier studies on the essential oils of *T. diffusa* revealed a total of Fifty-four components were characterized and identified, the most abundant being 1,8-cineol (11.4%), opoplenone (10.3%) cadalene (5.1%), and epi-cubenol (4.1%)[22].

The differences observed in the essential oils, phenolic and antioxidant contents of *T. diffusa* obtained from the different localities as reported by [23] suggested that climatic difference influence the phytochemicals in the plants and the essential oils yields could be associated with high environmental humidity.

In another study by[24], the leaf essential oil of *T. diffusa* were caryophyllene (43.7%) and germacrene B (21.3%), whilst the stem bark oil was primarily composed of geranial (50.7%) and neral (33.6%). The chemical components result from the research of [24], show similarities with that in this research. However, a lot of components reported in this worked were not mentioned in that report.

Turnera diffusa leaf oil extract showed the presence of α-cardinol, caryophyllene, α-pinene, caryophyllene oxide, σ-cadinene compared with the result obtained in the studies by[23]. In the study of the essential oil of *Turnera diffusa* by[22], 54 components were reported and identified with the most abundant compounds being 1,8-cineol (11.4%), opoplene (10.3%), cadalene (5.1%) and epi-cubeniol (4.1 %)

CONCLUSION

The GC-MS analysis revealed that the essential oils of *Turnera diffusa* leaf and stem are abundant in mono and sesquiterpenes. The essential oil of *T. diffusa* from this research showed a marked variation in composition of the leaf and steam oils. In comparison with other studies, it was observed that the chemical composition of essential oils depends on the method of extraction of the plant, climatic conditions, soil composition.

REFERENCES

1. Everitt, J.H., D.L. Drawe, R.I. Lonard. 2002. *Trees, Shrubs, and Cacti of South Texas*. Texas Tech University Press. p. 208. ISBN 978-0-89672-473-0.

2. Szewczyk, K. and C. Zidorn. 2014. "Ethnobotany, Phytochemistry, and Bioactivity of the Genus Turnera (Passifloraceae) with a focus on Damiana-*Turnera diffusa*". *J Ethnopharmacol*. 152(3): 424-443.

3. Johnson, T. 1999. *CRC Ethnobotany Desk Reference*. CRC Press: Boca Raton, FL.

4. Kumar, S., R. Taneja, and A. Sharm. 2006. “Pharmacognostic Standardization of *Turnera aphrodisiaca* Ward”. *J. Med. Food*. 9(2): 254–260.

5. Garza-Juárez, A., M. de la Luz Salazar-Cavazos, R. Salazar-Aranda, J. Pérez-Meseguer, and N.W. de Torres. 2011. “Correlation between Chromatographic Fingerprint and Antioxidant Activity of *Turnera diffusa* (Damiana)”. *Planta Med*. 77: 958–963.

6. Bernardo, J., I. Malheiro, R.A. Videira, P. Valentão, A.C. Santos, F. Veiga, and P.B.A. Trichiliacatigua. 2021. “*Turnera diffusa* Extracts: *In vitro* Inhibition of Tyrosinase, Antiglycation Activity, and Effects on Enzymes and Pathways Engaged in the Neuroinflammatory Process”. *J. Ethnopharmacol*. 271: 113865.

7. Estrada-Reyes, R., M. Carro-Juárez, and L. Martínez-Mota. 2013. “Pro-Sexual Effects of *Turnera diffusa* Wild (Turneraceae) in Male Rats involves the Nitric Oxide Pathway”. *Journal of Ethnopharmacology*. 146(1): 164-172. ISSN 0378-8741

8. Estrada-Reyes, R., P. Ortiz-López, J. Gutiérrez-Ortíz, and L.A.M. Mota. 2009. “*Turnera diffusa* Wild (Turneraceae) Recovers Sexual Behavior in Sexually Exhausted Males”. *J. Ethnopharmacol*. 123: 423–429.

9. Zhao, J., A.K. Dasmahapatra, S.I. Khan, and I.A. Khan. 2008. “Anti-Aromatase Activity of the Constituents from Damiana (*Turnera diffusa*)”. *J. Ethnopharmacol*. 120(3): 387–393.16. http://webbook.nist.gov/chemistry/(accessed 23 January, 2020)

10. Taha, M.M.E., M.S. Salga, H.M. Ali, M.A. Abdulla, S. Abdelwahab, and A.H.A. Hadi. 2012. “Gastroprotective Activities of *Turnera diffusa* Willd. ex Schult. Revisited: Role of Arbutin”. *J. Ethnopharmacol*. 141: 273–281.

11. del Carmen Avelino-Flores, M., M. del Carmen Cruz-López, F.E. Jiménez-Montejo, and J. Reyes-Leyva. 2015. “Cytotoxic Activity of the Methanolic Extract of *Turnera diffusa* Willd on Breast Cancer Cells”. *Journal of Medicinal Food*. 299-305.

12. See reference 10.

13. Báez-Parra, K.M., M. Soto-Beltrán, O. López-Cuevas, J. Basilio Heredia, L. Alcaraz-Meléndez, and M.A. Angulo-Escalante. 2019. “*In Vitro* Antimicrobial Activity of Methanolic and Hexanic Extracts of *Turnera diffusa* Against Common Urinary Pathogens”. *Revista Bio Ciencias*. 6(2): e670

14. Takebayashi, J., R. Ishii, J. Chen, T. Matsumoto, Y. Ishimi, and A. Tai. 2010. “Reassessment of Antioxidant Activity of Arbutin: Multifaceted Evaluation using Five Antioxidant Assay Systems”. *Free Radical Research*. 44: 473–478.

15. See reference 2.

16. Chevallier, A. 1996. *The Encyclopedia of Medicinal Plants*. Dorling Kindersley: London, UK.

17. See reference 4.

18. Njoku, I.O., O.T. Asekun, and O.B. Familoni. 2017. “Effects of Different Drying Methods on the Chemical Composition of the Essential Oils of *Ocimum gratissimum* L. Leaves”. *Nig J Pure Appl Sci*. 30(3): 3109-3115.

19. NIST LIBRARY. 2020. National Institute of Standards and Technology: Washington, DC. http://webbook.nist.gov/chemistry/ (accessed 22 January 2020).

20. El-Sayed, A.M. 2014. “The Pherobase: Database of Pheromones and Semiochemicals”. URL http://www. pherobase.Com.2012. (accessed 15 June, 2021).

21. Adams, R.P. 2007. “Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry”. Vol. 456. Allured Publishing Corporation: Carol Stream, IL. pp.804.

22. Sefidkon, F., A. Khadijeh, and B.K. Gholamreza. 2006. “Influence of Drying and Extraction Methods on Yield and Chemical Composition of the Essential Oil of *Satureja hortensis*”. *Food Chem*. 99(1):19-23.

23. Bicchi, C., P. Rubiolo, E.E. Saranz Camargo, W. Vilegas, J. de Souza Gracioso, and A.R. Monteiro Souza Brito. 2003. “Components of *Turnera diffusa* Willd. var. afrodisiaca (Ward) Urb. Essential Oil”. *Flavour Frag J*. 18(1): 59-61.

24. Alcaraz-Mel´endez, L., S. Real-Cosío, V. Suchý, and E. Svajdlenka. 2007. “Differences in Essential Oil Production and Leaf Structure in Phenotypes of Damiana (*Turnera diffusa* Willd)”. *J Plant Biol*. 50(3): 378-382.

25. Oladipupo, A.L., O.N. Avoseh, O.J. Tinubu, I.A. Ogunwande, R. Ascrizzi, G. Flamini, and F.M. Mtunzi. 2020. “Anti-Nociceptive Property, Anti-Inflammatory Activity and Constituents of Essential Oils from the Leaves and Stem Bark of *Turnera diffusa* Wild (Passifloraceae) Growing in Nigeria”. *Journal of Biologically Active Products from Nature*. 10(6): 473-483.

SUGGESTED CITATION

Njoku, I.S., M. Olaleye, and O.T. Asekun. 2022. "Comparative Study of Chemical Composition of the Essential Oils of *Turnera diffusa* (Josef August Schultes) Leaf and Stem". *Pacific Journal of Science and Technology*. 23(2): 101-106.

Pacific Journal of Science and Technology