

Medicinal Studies on the Phytochemical Constituents of *Justicia carnea* by GC-MS Analysis

I. E. Otuokere^{1, *}, A. J. Amaku¹, K. K. Igwe², G. C. Chinedum¹

¹Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Abia, Nigeria

²Department of Vet. Biochem and Pharm, Michael Okpara University of Agriculture, Umudike, Abia, Nigeria

Abstract

The phytochemical constituents of ethanol extracts of *Justicia carnea* leaf was analyzed using gas chromatography-mass spectrometry method. The ethanol leaf extracts of *Justicia carnea* was prepared by soxhlet extraction method and concentrated at 40°C using hot air oven. The concentrated ethanol extracts was subjected to phytochemical analysis using GC-MS. GC-MS analysis showed the presence of six phytochemicals. The phytochemicals are isonicotinic acid N-oxide (2.58%), phosphinodithioic acid, diphenyl- (1.93%), hexadecanoic acid (10.50%), 2,2,3,3,4,4,5,5,5-nonafluoro-pentanoic acid methyl ester (73.19%), 9,12,15-octadecatrien-1-ol (9.33%) and 7H-purine, 7-benzyl-2,6-dichloro- (2.48%). The result of the GC-MS analysis showed that the ethanol extract of *Justicia carnea* contains many pharmacologically important bioactive compounds. Traditionally, *Justicia* species are used in the treatment of inflammation, gastrointestinal diseases, respiratory tract infection, rheumatism and arthritis. There is need for isolation of these phytochemicals for the control of diseases.

Keywords

Justicia carnea, Gas Chromatography, Mass Spectrometry, Extracts, Phytochemicals

Received: June 18, 2016 / Accepted: June 30, 2016 / Published online: July 15, 2016

© 2016 The Authors. Published by American Institute of Science. This Open Access article is under the CC BY license.

<http://creativecommons.org/licenses/by/4.0/>

1. Introduction

Justicia carnea is a flowering plant that belongs to Acanthaceae family [1]. It is commonly called Brazilian plume flower, Brazilian-plume, flamingo flower and jacobinia. This flowering plant is a native of Atlantic Forest eco regions of eastern Brazil. It is also found in Nigeria. It is a decorative plant that is cultivated in warm temperate and subtropical climates. The genus *Justicia* was named after a Scottish gardener James Justice, in the 18th century [2]. The plant can be wintered indoors and lifted into pots. The shades of the plant during summer ranges from white, pink, red, rose, magenta, orange, purple to coral/apricot [2]. The flowers are tube like in shape and curves outward from the spike. The smaller varieties may grow about 2 feet high while others may grow to 6 feet tall by 6 feet wide [2]. The

pictorial view of *Justicia carnea* is shown in Figure 1.



Figure 1. Pictorial view of *Justicia carnea*.

* Corresponding author

E-mail address: ifeanyiotuokere@gmail.com (I. E. Otuokere)

Traditionally, *Justicia* species are used in the treatment of inflammation, gastrointestinal diseases, respiratory tract infection, rheumatism and arthritis [3]. Because of their effect on the central nervous system, they are utilized as hallucinogens, sedatives and treatments for epilepsy, depressors, somniferous agents and mental disorders [3]. Some species of *Justicia* are used in the treatment of diabetes, HIV and cancer [3]. The root, flower, fruit and leaf of *J. adhatoda* have been used in the treatment of bronchitis, cold, whooping cough, asthma, helminthic, diarrhoea, dysentery, and glandular tumor, expectorant, tuberculosis, abortifacient, microbial, tussive and cancer [4, 5]. In Benin, decoction of *J. anselliana* leaves and root are used in the treatment of heart diseases and testicles inflammation [6]. The constituents of *J. spicigera* are potassium, calcium acetate, oxalate, sulfate, sodium chloride, simple carbohydrates, mucilages, pectins, glycosides, pigments, resin and essential oils. Flavonoids have been isolated from the leaves [7]. Sepúlveda-Jiménez and co-workers reported the total phenols, flavonoids and antioxidant activity of *J. Spicigera* [8]. Euler and Adam [9] characterization based on NMR, GC-MS and UV analysis of *J. spicigera* extracts suggested the presence of trirhamnosides of kaempferol and kaempferitrin. Domínguez et al. [10] identified the presence of β -sitosterol, 3-O-glucoside of β -sitosterol and cryptoxanthin in hexane-isopropyl ether-methanol extracts of *J. spicigera*.

Presently, the interest in studying and understanding the constituents of plants with healing properties have increased. The interest is focused in obtaining biologically active molecules and using them for curing various diseases. In continuation with the ongoing research on phytochemicals of *Justicia* species, we hereby present GC-MS analysis of ethanol extract of *Justicia carnea*.

2. Methodology

2.1. Plant Materials

Fresh leaves of *Justicia carnea* was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.2. Preparation of Plant Extract

Justicia carnea was dried in a shady place for 10 days and pulverized to powder using electrical grinder. Extraction was performed using soxhlet method [11]. Thirty five grams (35 g) of powdered sample was introduced into the extraction chamber of the soxhlet extractor using ethanol as solvent at a temperature of 70°C for 48 hrs. At the end of the extraction, the extract was concentrated in an oven at 35°C. Dried extract was sent for GCMS analysis.

2.3. GCMS Analysis of *Justicia carnea*

The characterization of the Phytochemicals in *Justicia carnea* was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using aQP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5). The initial column temperature was 80°C for 1min, and then increased linearly at 70°C min⁻¹ to 220°C, held for 3 min followed by linear increased temperature 10°C min⁻¹ to 290°C for 10 min. The temperature of the injection port was 290°C and the GC-MS interface was maintained at 290°C. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min⁻¹. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

2.4. Identification of Phytochemicals in *Justicia carnea*

The retention indices, peak area percentage and mass spectra fragmentation pattern of GC-MS chromatogram of ethanol extract of *Justicia carnea* was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [12], WILEY8.LIB [13] and with published literature. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

3. Results

Gas chromatogram of the ethanol extract of *Justicia carnea* is presented in Figure 2.

The mass spectra data of *Justicia carnea* is shown in Figure 3.

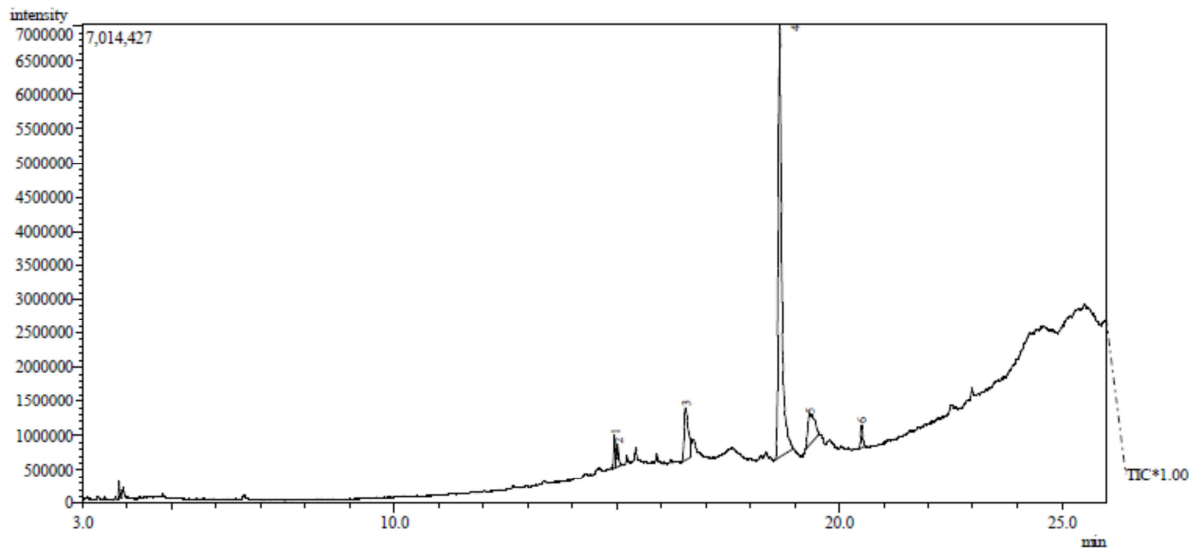


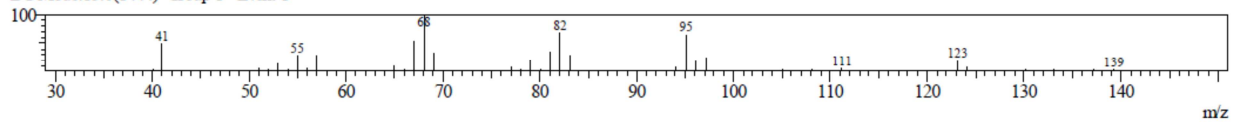
Figure 2. Gas Chromatogram of ethanol extract of *Justicia carnea* leaves.

Line# 1 R.Time:15.0(Scan#:1436)

MassPeaks:47

RawMode:Single 15.0(1436) BasePeak:68(33868)

BG Mode:15.0(1444) Group 1 - Event 1

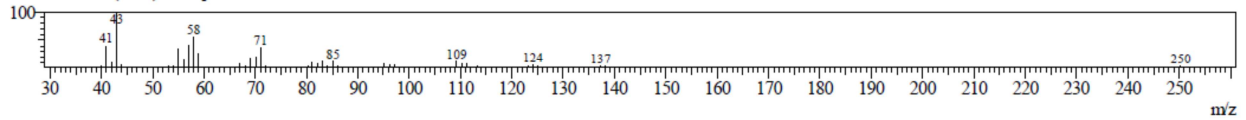


Line# 2 R.Time:15.0(Scan#:1444)

MassPeaks:61

RawMode:Single 15.0(1444) BasePeak:43(56028)

BG Mode:15.2(1459) Group 1 - Event 1

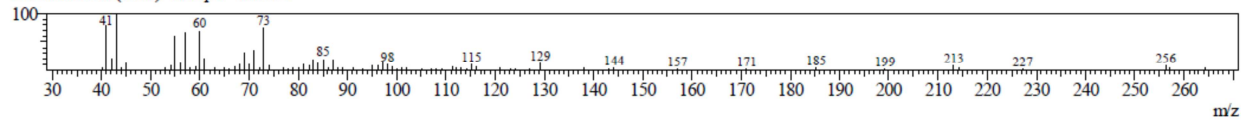


Line# 3 R.Time:16.6(Scan#:1627)

MassPeaks:89

RawMode:Single 16.6(1627) BasePeak:43(57770)

BG Mode:16.7(1641) Group 1 - Event 1

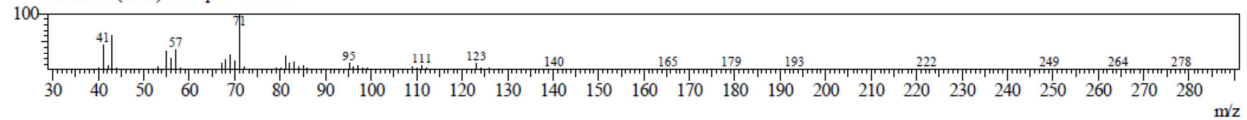


Line# 4 R.Time:18.7(Scan#:1881)

MassPeaks:87

RawMode:Single 18.7(1881) BasePeak:71(1177098)

BG Mode:19.1(1928) Group 1 - Event 1

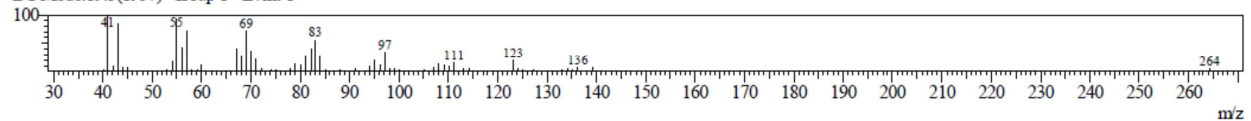


Line# 5 R.Time:19.3(Scan#:1960)

MassPeaks:66

RawMode:Single 19.3(1960) BasePeak:41(17339)

BG Mode:19.3(1957) Group 1 - Event 1



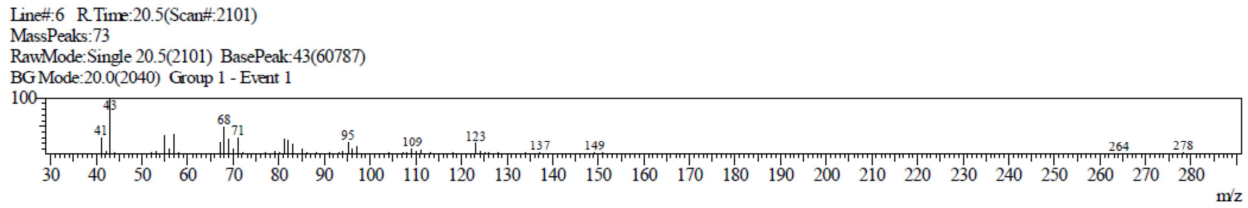


Figure 3. Mass spectra of the six phytochemicals in ethanol extract of *Justicia carnea*.

4. Discussion

The GC shows six peaks. This implies that six compounds are present in the ethanol extract of *Justicia carnea*. The mass spectra (Figure 3) were used in the identification of the molecular or parent ions. The molecular ion peak appears as the highest mass number in the mass spectrum and corresponds to

the molecular weight of the compound. This peak may sometimes be difficult to identify because it is usually of low intensity or may not appear at all. The names, retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities of the suggested compounds in the methanol extract of *Justicia carnea* are discussed in Table 1. The structures are shown in Figures 4–9.

Table 1. Name, retention time, peak area, molecular formula, molecular weight and bioactivity of phytochemicals in ethanol extracts of *Justicia carnea*.

S.No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula	Bioactivity
1	Isonicotinic acid N-oxide	14.96	2.58	139.10	C ₆ H ₅ NO ₃	Nitric-oxide-synthase-inhibitor, anaphylactic (antidote), arylamine-n-acetyltransferase-inhibitor, down regulation cytosol androgen [14].
2	Phosphinodithioic acid, diphenyl-	15.03	1.93	250.32	C ₁₂ H ₁₁ PS ₂	Acidifier, arachidonic-acid-inhibitor [14].
3	Hexadecanoic acid also known as Palmitic acid	16.55	10.50	256.42	C ₁₆ H ₃₂ O ₂	Acidifier, arachidonic-acid-inhibitor [14].
4	2,2,3,3,4,4,5,5,5-Nonafluoropentanoic acid methyl ester	18.67	73.19	278.07	C ₆ H ₃ F ₉ O ₂	Acidifier, arachidonic acid-inhibitor [14].
5	9,12,15-Octadecatrien-1-ol	19.34	9.33	264.44	C ₁₈ H ₃₂ O	Oligosaccharide provider [14].
6	7H-Purine, 7-benzyl-2,6-dichloro-	20.50	2.48	279.12	C ₁₂ H ₈ Cl ₂ N ₄	11β-HSD-inhibitor, 5-HT ₃ -inhibitor, antidote (heavy metals) [14].

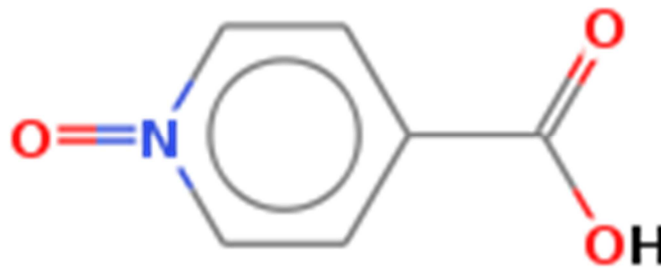


Figure 4. Isonicotinic acid N-oxide.

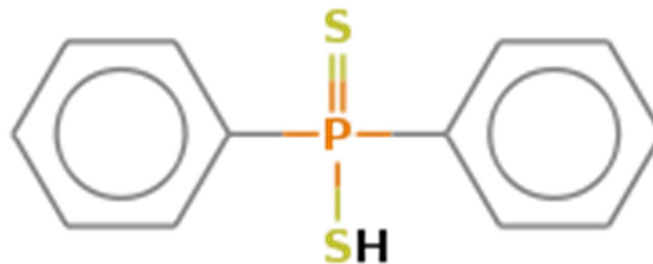


Figure 5. Phosphinodithioic acid, diphenyl-.

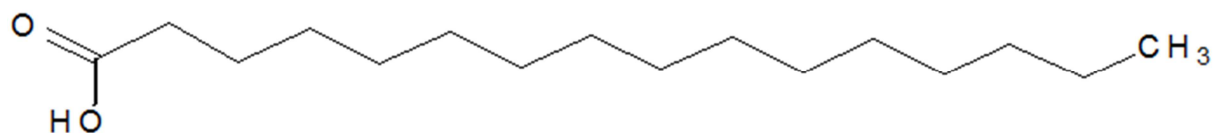


Figure 6. Hexadecanoic acid also known as Palmitic acid.

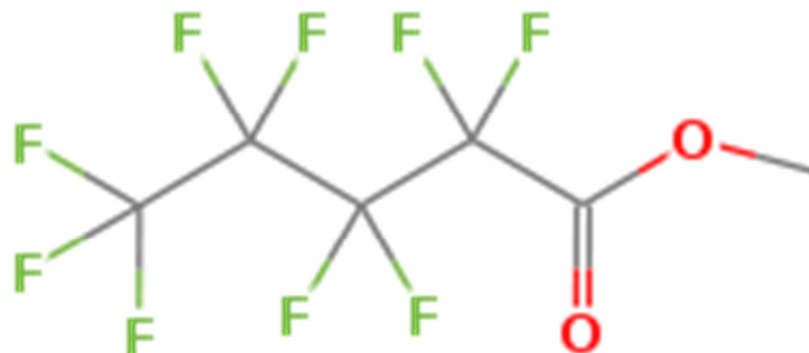


Figure 7. 2,2,3,3,4,4,5,5,5-Nonafluoro-pentanoic acid methyl ester.

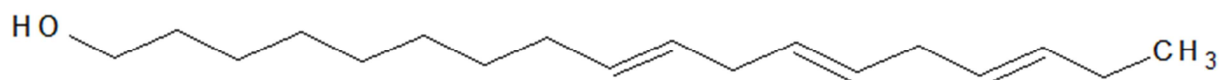


Figure 8. 9,12,15-Octadecatrien-1-ol.

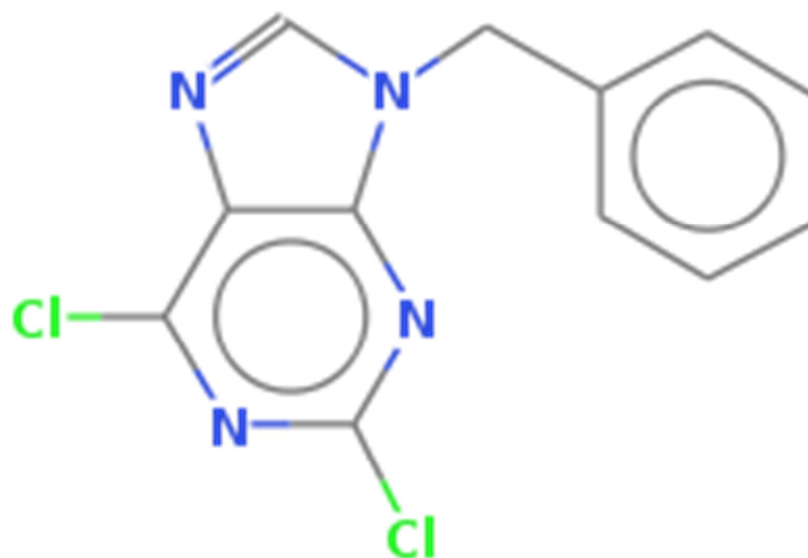


Figure 9. 7H-Purine, 7-benzyl-2,6-dichloro-.

Isonicotinic acid N-oxide (Figure 4) with retention time of 14.96 minutes and 2.58% peak area is a nitric-oxide-synthase-inhibitor. Its inhibition activity against nitric oxide is beneficial to man because nitric oxide is an air pollutant that causes cancer, inflammation and stroke. Researchers have shown that nitric oxide is a vascular relaxant [15-18] in the human body. Studies have shown that isonicotinic acid N-oxide is an anaphylactic antidote.

Anaphylaxis is a serious infection that might lead to death if not checked because its duration is within minutes to hours

[19]. It is an allergic reaction that is accompanied with low blood pressure, itching, swelling of the tongue, shortness of breath, vomiting, and light-headedness [21, 23]. This allergic reaction is triggered by insect bites, medication, food or any foreign substance [20, 22]. *Justicia carnea* ethanol extract might be a remedy to anaphylaxis because of presence of isonicotinic acid N-oxide (anaphylactic antidote) [14].

Arylamine N-acetyltransferases are highly conserved enzymes [24] that are genetically polymorphic to certain types of cancers. Numerous publications have shown that

some types of ArylamineN-acetyltransferases genotype have been associated with carcinogen exposure and cancer risk [25]. Isonicotinic acid N-oxide helps down regulation of cytosol androgen [14]. Prostate epithelial cells require androgen for growth [25]. Androgen binds to androgen receptors, which further binds to androgen-responsive elements for the promotion of androgen-regulated genes such as prostate-specific antigen (PSA). The down regulation of cytosol androgen provides an important mechanism in prostatic cancer chemoprevention [26].

Phosphinodithioic acid diphenyl- (Figure 5), Hexadecanoic acid (Figure 6) and 2,2,3,3,4,4,5,5,5-Nonafluoro-pentanoic acid methyl ester (Figure 7) have been reported to be acidifier and arachidonic acid inhibitor [14]. Acidifiers are chemicals that reduce the pH of the body. They help in food digestion in patients suffering from achlorhydria. These patients are not able to secrete HCl for food digestion. These compounds may be beneficial since they increase gastric acid when ingested. Arachidonic acid is present in the brain, muscles, and liver [28]. Arachidonic acid is a fatty acid that is polyunsaturated in nature and responsible for the repair and growth of skeletal body tissue [29]. Does not cause cancer but studies have proven that it might be a major cause of inflammation [30-33].

9,12,15-Octadecatrien-1-ol (Figure 8), one of the isolates of *Justicia carnea* with molecular weight 264.44 and peak area 9.33% is an oligosaccharide provider [14]. It helps in cell division and cell binding. It also improves gastrointestinal health, energy levels and performance. Oligosaccharide provider simply means little sugar [34].

The primary activity of 11 β -hydroxysteroid dehydrogenase (11 β -HSD-Inhibitor) type 1 is to reduce cortisone to the active hormone cortisol. Too much of cortisol in the human body leads to obesity [35]. 7H-Purine, 7-benzyl-2,6-dichloro- (Figure 9) has the tendency to reduce cortisone to cortisol. 5-HT₃ receptor antagonists are widely used chemotherapy antiemetic drugs [36]. They were first introduced in 1990 and have proven to be effective and safe against postoperative nausea and vomiting [37]. Our report suggested that 7H-Purine, 7-benzyl-2,6-dichloro-, a constituent of *Justicia carnea* is an antagonist of 5-hydroxytryptamine (5-HT₃) receptors. 7H-Purine, 7-benzyl-2,6-dichloro- also acts as an antidote for heavy metal. This compound can chelate heavy metals because it contains lone pairs of electrons that can act as electron donors. Heavy metals have empty d-orbitals onto which electrons may be promoted.

GC-MS is an effective method in the characterization of plant secondary metabolites. Several authors have used this method in the identification of bioactive compounds [38-43]. Hence, GC-MS analysis is a pioneer step in understanding the constituents of plants.

5. Conclusion

In this study, six compounds were identified in the ethanol extract of *Justicia carnea*. These identified phytochemicals may be helpful for drug development. This study may also enhance the traditional usage of *Justicia carnea* due to its bioactive compounds identified by GC-MS analysis. These phytochemicals need further pharmacological investigation in order to develop new drugs for the treatment of specific diseases. Thus, GC-MS analysis is the pioneer step in understanding the nature of active components in *Justicia carnea*.

References

- [1] "Justicia carnea". Natural Resources Conservation Service PLANTS Database. USDA.
- [2] <http://www.gaygardener.com>
- [3] Badami, S., Aneesh, R., Sankar, S., Sathishkumar, M. N., Suresh, B., Rajan, S. (2003). Antifertility activity of *Derris brevipes* variety coriacea. *J Ethnopharmacol* 84: 99-104.
- [4] Aswal, B. S., Bhakuni, D. S., Goel, A. K., Kar, K., Mehrotra, B. N., Mukherjee, K. C. (1984). Screening of Indian plants for biological activity: Part X. *Indian J Exp Biol*, 22: 312-332.
- [5] Joshi AR, Joshi K 2000. Indigenous knowledge and uses of medicinal plants by local communities of the Kali Gandaki Watershed Area, Nepal. *J Ethnopharmacol*, 73: 175-183.
- [6] Kpoviéss, D. S. S., Gbaguidi, F., Gbénoua, J., Accrombessi, G., Moudachiroua, M., Rozet, E., Hubert, P., Quetin-Leclercq, J. (2008). Validation of a method for the determination of sterols and triterpenes in the aerial part of *Justicia anselliana* (Nees) T. Anders by capillary gas chromatography. *J Pharmaceut Biomed*, 48: 1127-1135.
- [7] Osuna-Torres, L., Tapia-Pérez, M. E., Aguilar-Contreras, A. (2005). *Justicia spicigera* Schldl. (Acanthaceae) as a medicinal plant in the treatment of gastro intestinal infections in Mexico. Barcelona, España: *Publicacions Edicions de la Universitat de Barcelona*.
- [8] Sepúlveda-Jiménez, G., Reyna-Aquino, C., Chaires-Martínez, L., Bermúdez-Torres, K., Rodríguez-Monroy, M. (2009). Antioxidant Activity and Content of Phenolic Compounds and Flavonoids from. *J Biol Sci*, 9 (6):629-632.
- [9] Euler, K. L., Adam, M. (1982). Isolation of Kaempferitrin from *Justicia spicigera*. *J Nat Prod*, 45: 220-222.
- [10] Domínguez, X. A., Achenbach, H., González, C., Ferré-D'Amare, A. (1990). Estudio Químico del "Muitle" (*Justicia spicigera*). *Rev Latinoam Quim* 1990, 21(3-4): 142-143.
- [11] Jensen, W. B. (2007) The origin of Soxhlet Extraction. *Journal Clinical Education*. 84 (12), 1913-1914.
- [12] Stein, S. E. (1990). National Institute of Standards and Technology (NIST), Mass Spectral Database and Software, Version 3.02, USA.
- [13] Mc Lafferty, F. W. (1986). Registry of mass spectral data. Fourth electronic ed. Wiley New York.

- [14] Dr. Duke's Phytochemical and Ethnobotanical Databases, (1992 -1996). U. S. Department of Agriculture, Agricultural Research Service. <http://phytochem.nal.usda.gov>
- [15] Katsuki, S., Arnold, W., Mittal, C. and Murad, F. (1977). Stimulation of guanylatecyclase bisodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucleotide Res.* 3, 23-35.
- [16] Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. and Chaudhuri, G. (1987) Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA* 84, 9265-9269.
- [17] Khan, M. T. & Furchgott, R. (1987). Additional evidence that endothelium-derived relaxing factor is nitric oxide, Elsevier, Amsterdam.
- [18] Palmer, R. M. J., Ferrige, A. G. and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 327, 524-526.
- [19] "Anaphylaxis", (2016), National Institute of Allergy and Infectious Diseases.
- [20] Simons, F. E. (2010). "World Allergy Organization survey on global availability of essentials for the assessment and management of anaphylaxis by allergy-immunology specialists in health care settings". *Annals of Allergy, Asthma & Immunology*, 104 (5): 405–12.
- [21] Lee, J. K., Vadas, P. (2011). "Anaphylaxis: mechanisms and management". *Clinical and Experimental Allergy* 41 (7): 923–938.
- [22] Boden, S. R., Wesley, B. A (2011). "Anaphylaxis: a history with emphasis on food allergy". *Immunological Reviews*, 242 (1): 247–257.
- [23] Worm, M. (2010). "Epidemiology of anaphylaxis". *Chemical Immunology and Allergy*, 95: 12–21.
- [24] Butcher, N. J., Boukouvala, S., Sim, E., Minchin, R. F. (2002). Pharmacogenetics of the arylamine N-acetyltransferases. *Pharmacogenomics J*, 2: 30–42.
- [25] Hein, D. W. (2000). N-Acetyltransferase genetics and their role in predisposition to aromatic and heterocyclic amine-induced carcinogenesis. *ToxicolLett*, 112–113: 349–356.
- [26] Isaacs, J. T. (1994). Role of androgens in prostatic cancer. *Vitam Horm*; 49: 433–502.
- [27] Trapman, J., Cleutjens, K. B. (1997). Androgen-regulated gene expression in prostate cancer. *Semin Cancer Biol*, 8: 29–36.
- [28] Smith, G. I. Atherton, P., Reeds, D. N. Mohammed, B. S., Rankin, D., Rennie, M. J., Mittendorfer, B. (2011). "Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women." *Clinical science*, 121 (6): 267–78.
- [29] Trappe, T. A., Fluckey, J. D., White, F., Lambert, C. P., Evans, W. J. (2001). "Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen". *The Journal of Clinical Endocrinology and Metabolism*, 86(10): 5067–70.
- [30] Schuurman, A. G., Van Den Brandt, P. A., Dorant, E., Brants, H. A., Goldbohm, R. A. (1999). "Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study". *Cancer* 86 (6): 1019–1027.
- [31] Leitzmann, M. F., Stampfer, M. J., Michaud, D. S., Augustsson, K., Colditz, G. C., Willett, W. C., Giovannucci, E. L., (2004). "Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer". *The American Journal of Clinical Nutrition*, 80 (1): 204–216.
- [32] Astorg, P. (2005). "Dietary fatty acids and colorectal and prostate cancers: epidemiological studies". *Bulletin du cancer*, 92 (7): 670–684.
- [33] Whelan, J., McEntee, M. F. (2004). "Dietary (n-6) PUFA and intestinal tumorigenesis". *The Journal of Nutrition*, 134 (12): 3421–3426.
- [34] Walstra, P., Wouters J. T. M. and Geurts, T. J. (2008). Dairy Science and Technology, second edition. CRC, Taylor & Francis.
- [35] Nixon, M., Wake, D. J., Livingstone, D. E., Stimson, R. H., Esteves, C. L., Seckl, J. R., Chapman, K. E., Andrew, R., Walker, B. R. (2012). "Salicylate downregulates 11 β -HSD1 expression in adipose tissue in obese mice and in humans, mediating insulin sensitization". *Diabetes*, 61 (4): 790–6.
- [36] Brunton, L. L., Lazo, J. S., Parker, K. L. (2006). *Godman & Gilman's the Pharmacological Basis of Therapeutics*. New York: McGraw-Hill. 1000–1003.
- [37] Gan, T. J. (2005). "Selective serotonin 5-HT₃ receptor antagonists for postoperative nausea and vomiting: are they all the same?". *CNS Drugs*. 19 (3): 225–38.
- [38] Igwe, K. K., Nwankwo, P. O., Otuokere, I. E., Ijioma, S. N., Amaku, F. J., Chika, I. (2015). Identification of the phytochemicals in *Loranthus micranthus* methanolic extract by gas chromatography – mass spectrometry, *International Journal of Research Science and Management*, 2(12): 53–57.
- [39] Igwe, K. K., Nwankwo, P. O., Otuokere, I. E., Ijioma, S. N., Amaku, F. J. (2015). GC-MS analysis of phytochemicals in the methanolic extract of *Moringa Oleifera* leave, *Journal of Research in Pharmaceutical science*, 2(11): 01–06.
- [40] Igwe, K. K., Nwankwo, P. O., Otuokere, I. E., Chika, I., Amaku, F. J. (2016). Studies on the medicinal plant *Acalypha Wilkesiana* ethanol extract phytochemicals by GC-MS analysis, *Global Journal of Science Frontier Research*, 16(2): 48–55.
- [41] Igwe, K. K., Madubuike, A. J., Chika, I., Otuokere, I. E., Amaku, F. J. (2016). Studies on the medicinal plant *Pausinystalia yohimbe* ethanol leaf extract phytochemicals by GC-MS analysis, *International Journal of Scientific Research and Management*, 4(5): 4116–4122.
- [42] Igwe, K. K., Madubuike, A. J., Otuokere, I. E., Amaku, F. J., Chika, I. (2016). GC-MS analysis for structural identification and bioactive compounds in methanolic leaf extract of *Mallotus oppositifolius*, *International Journal of Scientific Research and Management*, 4(5): 4123–4128.