Characterization of Calopogunium mucunoides Ethanol Extract by Gas Chromatography–Mass Spectrometry Analysis

D. O. Okorie[1], I. E. Otuokere[1], K. K. Igwe[2], C. Azubuike[1]

[1]Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria
[2]Department of Vet. Biochem and Pharm, Umudike, Nigeria

[1]ifeanyiotuokere@gmail.com
[2]tosmanbaba@yahoo.com

Abstract— ***Calopogunium mucunoides*** **are forage leguminous plants that grows annually or perennially. The leaves of *Calopogunium mucunoides* are used for the management of ulcer. Gas chromatography-mass spectrometry method was used in the characterization of *Calopogunium mucunoides*. The extract was prepared using soxhlet extraction method and concentrated at 40ºC in an oven. The concentrated extract was analyzed using GC-MS analysis. The gas chromatogram showed the presence of twelve compounds. The suggested compounds are histamine (1.60%), 2-ethyl-2-hexen-1-al (2.45%), methenamine (1.86%), 9,12,15-octadecatrien-1-ol (6.26%), methyl 14-methylpentadecanoate (8.01%), hexadecanoic acid (6.18%), hexadecanoic acid, ethylester (9.13%), 9,12-octadecadienoic acid, methylester (34.98%), 2-ethylhex-3-enal (16.13%), 1H-Indol-5-ol (2.73%), p-methoxybenzoic acid, octylester (3.97%), gamolenic acid (6.70%). Bioactivity studies showed that *Calopogunium mucunoides* is an acidifier, acidulant, histamine-Inhibitor, oligosaccharide provider, catechol-o-methyl-transferase-inhibitor, inhibitor of uric acid production, antidote (heavy metals) and previtamin-A provider. We hereby recommend the isolation and synthesis of these bioactive compounds for drug development.**

Keywords— **Calopogunium mucunoides, gas chromatography, mass spectrometry, phytocompounds, ethanol, retention time**

I. INTRODUCTION

Medicinal plants can be defined as plants that contain secondary metabolites and have been used for the treatment of ailments [1]. Some authors believe that for a plant to be term "medicinal", its bioactivity must have been published [2]. In Africa, plants are used traditionally as medicine for the management for diseases. According to WHO [3], 90% of the developing countries use plant to meet their primary health care needs. It has been reported [4] that 80% of the world population use plant for the treatment of diseases. The use of plant is not only limited to the third world countries. Developed countries use modern pharmacopeia which has plant as origin. The primary metabolites are beneficial to plant while the secondary metabolites are important to man [5].

Calopogunium mucunoides (Figure 1) are forage leguminous plants that grow annually or perennially. It grows to several metres but usually shallow rooted, about 30 cm deep. It forms a tangle mass of foliage that twins, and creeps vigorously at a depth of 30 - 40 cm deep. Trifoliate leaves with small triangular stipules, short flowers and linear pods compressed to 2.5 – 4.0 cm long [6]. In Thailand, the duration of flowering and fruiting of Calopogunium mucunoides is from December to March [7]. In Nigeria, flowering and fruiting is usually during the rainy season (April to October). It is naturalized in Australian, Asia, Africa and the Pacific Island [8]. Its seed are quadrangular, about 3 mm in width, shiny and reddish brown [7]. Calopogunium mucunoides has been described to be hermaphroditic in nature [9].

Figure 1: Pictorial view of *Calopogunium mucunoides*

The leaves of Calopogunium mucunoides are used for the management of ulcer in South Eastern Nigeria [10]. Oral administration of the ethanol extract of the leaves of Calopogonium mucunoides to Wistar rats caused a substantial gastric cytoprotection against the ethanol-induced ulcer in the rats and therefore, makes the plant a possible future candidate for refined anti-ulcer drug(s) [10].Decoction from the leaves are used for strengthening the system and as an anti-scorbutic. Leaves are used in the treatment of diarrhea [11]. Mixture of Calopogunium mucunoides and palm oil are used in the eradication of measles and chicken pox. Mixture of Calopogunium mucunoides leaves decoction and wood ash are efficient in the treatment of dysentery. The bark, when mixed with palm wine produces tonic effect [11]. In continuation with the ongoing research on Calopogunium mucunoides, we have decided to characterize the bioactive phytocompounds of Calopogunium mucunoides ethanol extract by GC-MS analysis.

II. METHODOLOGY

Plant Materials

Fresh leaves of Calopogunium mucunoides was harvested in Ogbuebulle, Ikwuano, Abia State, Nigeria. Identification of the plant leaves was done at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract

Calopogunium mucunoides was dried in a shady place for 10 days and pulverized to powder using electrical grinder. Extraction was performed using Soxhlet method [12]. 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 70o C for 48 hrs. At the end of the extraction, the extract was concentrated in an oven at 35oC. Dried extract was sent for GCMS analysis.

GC-MS analysis of Calopogunium mucunoides

The characterization of the phytochemicals in Calopogunium mucunoides was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 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 80oC for 1min, and then increased linearly at 70oC min-1 to 220oC, held for 3 min followed by linear increased temperature 10oC min-1 to 290oC for 10 min. The temperature of the injection port was 290oC and the GC-MS interface was maintained at 290oC .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.

Identification of Phytocompoments in Calopogunium mucunoides

The retention indices, peak area percentage and mass spectra fragmentation pattern of GC-MS chromatogram of ethanol extract of Calopogunium mucunoides was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [13], WILEY8.LIB [14] and with published literature.

III. RESULTS

Gas chromatogram of the ethanolic extract of Calopogunium mucunoides is presented in Figure 2. The mass spectra data of Calopogunium mucunoides is show in Figure 3.

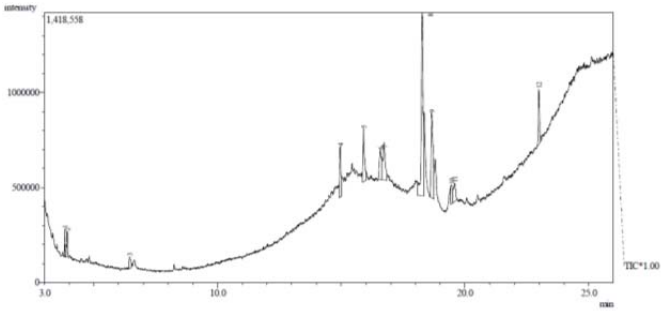
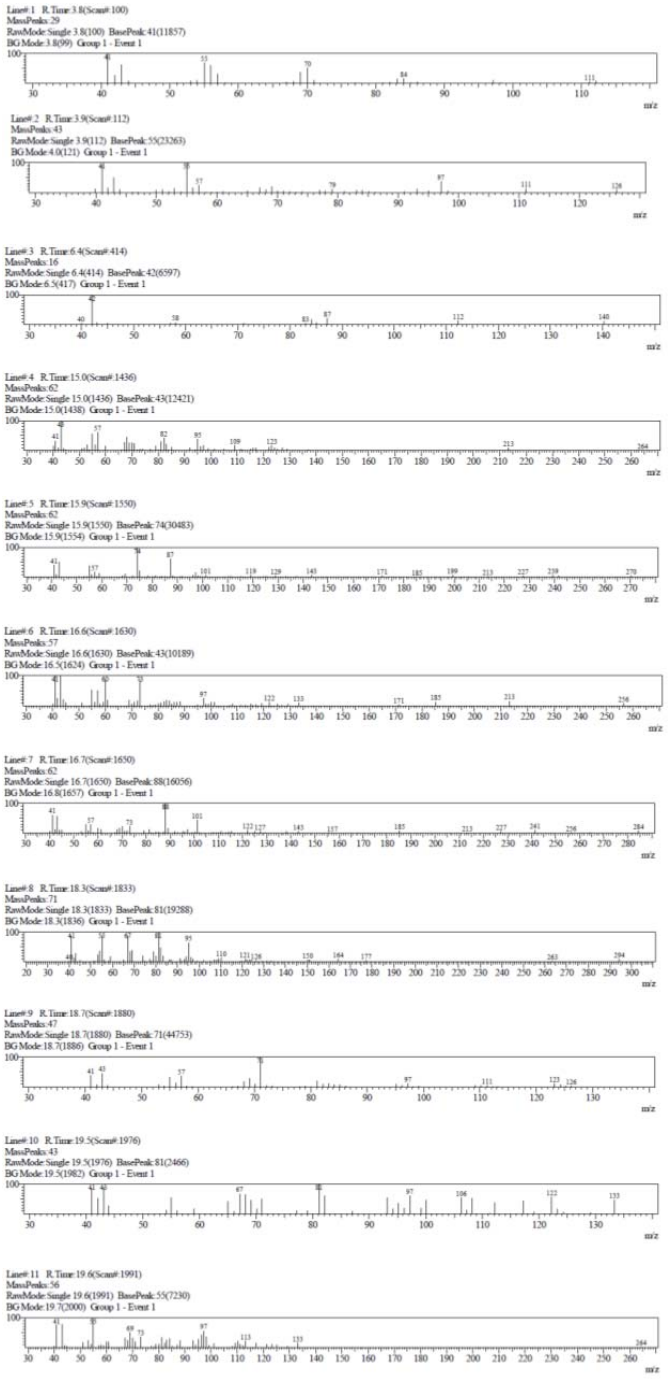


Fig 1 Gas chromatogram of *Calopogunium mucunoides* ethanol extract.



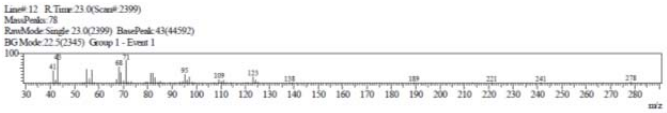


Fig. 2 Mass spectra of *Calopogunium mucunoides* ethanol extract

IV. DISCUSSION

Twelve peaks are shown in the gas chromatogram. These peaks suggest that twelve compounds are present in Calopogunium mucunoides ethanol extract. The compound names, retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities of the suggested compounds in the ethanol extract of Calopogunium mucunoides are disscussed in Table 1. The structures are shown in Figures 4 -15 respectively.

Calopogunium mucunoides contains histamine with a peak area of 1.60%. Antihistamines are compounds that inhibit histamine receptors in the body. They are used in the treatment of allergic nose reactions, insomnia, motion sickness, peptic ulcers and acid reflux. Calopogunium mucunoides leaf ethanol extract may act as histamine inhibitor because it contains histamine.

Hexadecanoic acid and hexadecanoic acid, ethyl ester have been reported to be acidifier, acidulant and arachidonic acid inhibitor [15]. 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 secret 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 [16]. Arachidonic acid is a fatty acid that is polyunsaturated in nature and responsible for the repair and growth of skeletal body tissue [17]. Arachidonic acid does not cause cancer but studies have proven that it might be a major cause of inflammation [18 -21]. Additives that reduce the pH of food in order to add a tart taste and characteristic tang are called acidulants. They also have preservative and antioxidative properties [22].

Catechol-o-methyl-transferase is involved in the degradation of neurotransmitters. 9,12-Octadecadienoic acid, methyl ester , a catechol-o-methyl-transferase-inhibitors opposes the degradation of neurotransmitters. Parkinson's disease is treatable with catechol-o-methyl-transferase-inhibitors [23].

9, 12, 15-Octadecatrien-1-ol, one of the isolates of Calopogunium mucunoides with molecular weight 264.44 and peak area 19.52% , is an oligosaccharide provider [15]. It helps in cell division and cell binding. It is also improves gastrointestinal health, energy levels and performance. Oligosaccharide provider simply means little or few sugar [24].

Calopogunium mucunoides phytoconstituent, 1H-Indol-5-ol is an antidote for heavy metals[15]. The ability of this phytocompound to eliminate heavy metal lies on its lone pair of electrons in –OH and –NH. These functional groups can chelate metal ions to form a chelated complex. These complexes are excreted out of the body. Hence, Calopogunium mucunoides can be used for chelation therapy.

Vitamin A is essential for healthy vision, immune system function and cell growth. It works synergetically with viamin D, K2 and zinc. Vitamin A can be categorized as retinoids and carotenoids. Tthe bioavailable forms of vitamin A is retinoids and it is found in animal food while carotenoids, previtamin A is found in plant food. Previtamin A must be converted to bioavailable retinol for body metabolism [25].

Uric acid is produced when purine nucleotides breaks down. High concentration of uric acid in the blood can lead to gout, diabetes and formation of ammonium acid urate kidney stones. Calopogunium mucunoides may help in the inhibition of uric acid because it contains gamolenic acid and hexadecanoic acid [26].

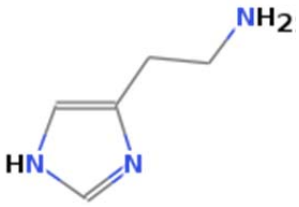


Figure 4:Histamine

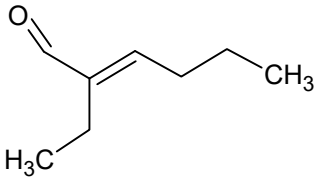


Figure 5: 2-Ethyl-2-hexen-1-al

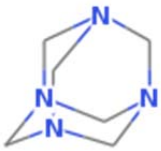


Figure 6: **Methenamine**

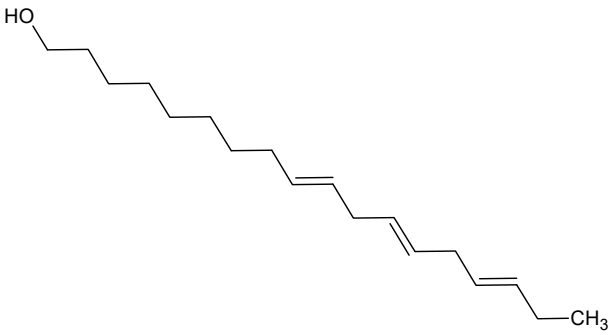


Figure 7: 9,12,15-Octadecatrien-1-ol

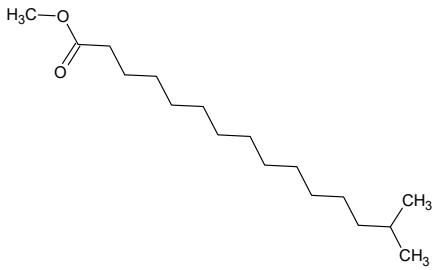


Figure 8: Methyl 14-methylpentadecanoate

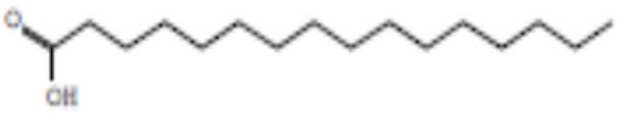


Figure 9: Hexadecanoic acid

Figure 10: Hexadecanoic acid, ethyl ester

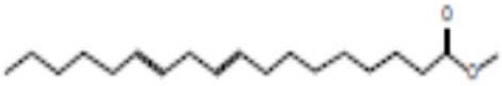


Figure 11: 9,12-Octadecadienoic acid, methyl ester

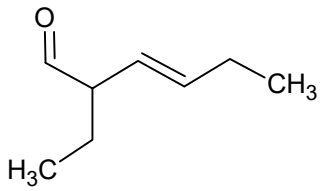


Figure 12: 2-ethylhex-3-enal

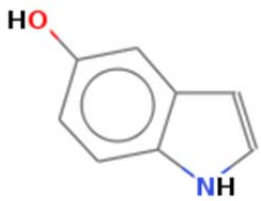


Figure 13: 1H-Indol-5-ol

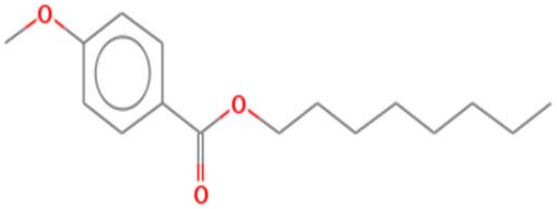


Figure 14: p-Methoxybenzoic acid, octyl ester

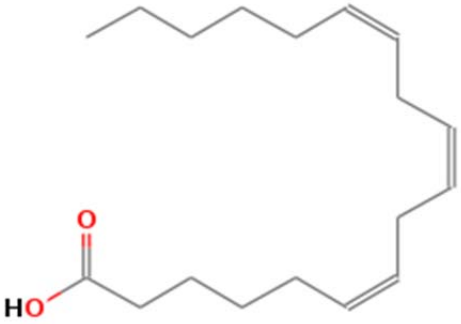


Figure 15: Gamolenic acid

TABLE I
NAMES, RETENTION TIME, PEAK AREA PERCENTAGE, MOLECULAR WEIGHT, MOLECULAR FORMULA AND BIOACTIVITIES

S/No	Name of Compound	Retention time	Peak area (%)	Molecular weight	Molecular formula	Bioactivity
1	Histamine	3.827	1.60	111.14	$C_5H_9N_3$	Histamine-Inhibitor
2	2-Ethyl-2-hexen-1-al	3.928	2.45	126.19	$C_8H_{14}O$	Not found
3	Methenamine	6.445	1.86	140.18	$C_6H_{12}N_4$	Not found
4	9,12,15-Octadecatrien-1-ol	14.955	6.26	264.44	$C_{18}H_{32}O$	Oligosaccharide Provider,
5	Methyl 14-methylpentadecanoate	15.903	8.01	270.45	$C_{17}H_{34}O_2$	Catechol-O-Methyl-Transferase-Inhibitor
6	Hexadecanoic acid also known as Palmitic acid	16.582	6.18	256.42	$C_{16}H_{32}O_2$	Inhibit Production of Uric Acid, acidifier.
7	Hexadecanoic acid, ethyl ester	16.727	9.13	284.47	$C_{18}H_{36}O_2$	Acidulant, acidifier
8	9,12-Octadecadienoic acid, methyl ester	18.269	34.98	294.47	$C_{19}H_{34}O_2$	Catechol-O-Methyl-Transferase-Inhibitor
9	2-ethylhex-3-enal	18.659	16.13	126.19	$C_8H_{14}O$	Not found
10	1H-Indol-5-ol	19.452	2.73	133.14	C_8H_7NO	Antidote (Heavy Metals)
11	p-Methoxybenzoic acid, octyl ester	19.583	3.97	264.36	$C_{16}H_{24}O_3$	Previtamin-A
12	Gamolenic acid ; 6,9,12-Octadecatrienoic acid; γ-Linolenic acid	22.985	6.70	278.42	$C_{18}H_{30}O_2$	inhibit Production of Uric Acid

V. CONCLUSIONS

GC-MS analysis of Calopogunium mucunoides showed the presence of twelve phytocompounds. Bioactivity studies showed that Calopogunium mucunoides is an acidifier, acidulant, histamine-inhibitor, oligosaccharide provider, catechol-o-methyl-transferase-inhibitor, inhibitor of uric acid production, antidote (heavy metals) and previtamin-A provider. We hereby recommend the isolation of these active ingredients for the treatment of diseases.

REFERENCES

[1] A. Sofowora, Medicinal Plants and Traditional Medicine in Africa, 2nd Edition. Spectrum Books Ltd, Ibadan, Nigeria, 1993

[2] A. A. Elujoba, O.M. Odeleye, C.M. Ogunyemi, "Traditional Medical Development for Medical and Dental primary health care delivery system in Africa". Afr. J. Trad. CAM., vol.2 (1), pp. 46-61, 2005

[3] WHO, Traditional medicine – growing needs and potentials. WHO Policy Perspectives Med. 2002

[4] WHO, Legal Status of Traditional Medicine and Complementary/Alternative medicine: A world-wide review. WHO Publishing 1, 2001

[5] G.E. Trease and W.C. Evans. Textbook of Pharmacognosy. 14th Edition W.B. Sanders, London, 1989.

[6] (2016) Cherukutty Sons website. [Online]. Available: http://www.cherukuttysons.In/Calopogonium mucunoides_covercrop_seeds.html.

[7] P. Acevedo-Rodríguez, Vines and climbing plants of Puerto Rico and the Virgin Islands. Contributions from the United States National Herbarium,vol. 51:483, 2005

[8] B. Cook, B. Pengelly, S. Brown , J. Donnelly, D. Eagle Franco, J. Hanson , B. Mullen, I. Partridge, M.Peters, R. Schultze-Kraft (2005). Tropical Forages: an interactive selection tool. Brisbane, Australia. [Online]. Available: http://www.tropicalforages.info/

[9] FAO (2013). Grassland Species Profiles. Detailed decription of more than 600 grassland species.Available: http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Default.html

[10] C.E. Osmund, E.O. Christian and C. Okafor, "Assessment of the anti-ulcer action of the leaves of calopo (Calopogonium mucunoides Desv) in Wistar rats", Journal of Pharmacy Research, vol. 8(1), pp. 24- 27, 2014

[11] T. I. Borokini and F. O. Omotayo, "Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria", Journal of Medicinal Plants Research, vol.6(7), pp.1106-1118, 2012

[12] W.B. Jensen, "The origin of Soxhlex Extraction" Journal Clinical Education. vol.84 (12), pp. 1913-1914, 2007.

[13] S.E. Stein, National Institute of Standards and Technology (NIST), Mass Spectral Database and Software, Version 3.02, USA, 1990.

[14] F.W. Mc Lafferty, Registry of mass spectral data. Fourth electronic ed. Wiley New York.

[15] Dr. Duke's Phytochemical and Ethnobotanical Databases (1992 –1996), U.S. Department of Agriculture, Agricultural Research Service. Available: http://phytochem.nal.usda.gov.

[16] G.I. Smith, P. Atherton, D.N. Reeds, B.S. Mohammed, D. Rankin, M.J. Rennie, B. Mittendorfer, "Omega-3 polyunsaturated fatty acidsaugment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women." Clinical science, vol.121 (6), pp. 267–278, 2011.

[17] T.A. Trappe, J.D. Fluckey, F. White, C.P. Lambert, W.J. Evans."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,vol. 86(10), pp. 5067–5070, 2001

[18] A.G. Schuurman, P.A. Van Den Brandt, E. Dorant, H.A. Brants, R.A. Goldbohm(1999). "Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study". Cancer, 86(6): 1019 – 1027.

[19] M.F. Leitzmann, M.J. Stampfer, D.S. Michaud, K. Augustsson, G.C. Colditz, W.C. Willett, E. L. Giovannucci, "Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer". The American Journal of Clinical Nutrition, vol.80(1), pp. 204 – 216, 2004

[20] P. Astorg (2005). "Dietary fatty acids and colorectal and prostate cancers: epidemiological studies". Bulletin du cancer, vol.92 (7), pp. 670 – 68, 2005.

[21] J. Whelan, M.F. McEntee, "Dietary (n-6) PUFA and intestinal tumorigenesis". The Journal of Nutrition, vol. 134(12): pp.3421 – 3426, 2004

[22] Food additives and ingredients association [Online]. Available: http://www.faia.org.uk/acidulant_in_food

[23] (2016).Wikipedia, the free encyclopedia [Online]. Available: http://en.wikipedia.org/wiki/COMT_inhibitors

[24] P. Walstra, J.T.M. Wouters and T.J. Geurts, Dair Science and Technology, second edition. CRC, Taylor & Francis. 2008

[25] (2016) Wikipedia, the free encyclopedia [Online]. Available: http://www. en.wikipedia.org/wiki/uric_acod

[26] (2016) The worlds healthiest foods. [Online]. Available http://www.worldhealthiestfood.com