Chapter 11

References

Ahmad, I. and Beg, A.Z. 2001.Antimicrobial and phytochemical studies of 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*. **74**: 113-123**.**

Akerele, O. 1993. Nature's medicinal bounty: don't throw it away. *World Health Forum*. **14**: 390-395.

Anesini, C. And Perez, C. 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal of Ethnopharmacology*. **39**: 119-128.

Begue, W.J. and Kline, R.N. 1972. The use of tetrazolium salts in bioautographic procedures Journal of Chromoatography. **64**: 182-184.

Blood, D.C., Henderson, J.A. and Radostits, O.M. 1979. *Veterinary Medicine* – A Textbook of the Diseases of Cattle, Sheep, Pigs and Horses, 5th edition. London: Bailliere Tindell.

Booth, N.L., Overk, C.R., Yao, P., Totura, S., Deng, Y., Hedayat, A.S., Bolton, J.L., Pauli, G.F. and Farnsworth, N.R. 2006. Seasonal variation of red clover (*Trifolium pratense* L., *Fabaceae*) isoflavones and estrogenic activity. *Journal of Agriculture and Food Chemistry*. **22**: 1277-1282.

Bossard, E. 1993. Angolan medicinal plants used also as piscicides and/or soaps. *Journal of Ethnopharmacology*. **40**: 1-19.

Bryant, A.T. 1966. *Zulu Medicine and medicine-men*. Cape Town: C.Struik.

Chopra, R.N., Nayer, S.L. and Chopra, I.C. 1992.*Glossary of Indian MedicinalPlants*, 3rd edition... Council of Scientific and Industrial Research, New Delhi, India.

Dalziel, J.M. 1937. *The Useful Plants of West Tropical Africa*. London: The Crown Agents.

Desta, B. 1993. Ethiopian traditional herbal drugs. Part II: Antimicrobial activity of 63 medicinal plants. *Journal of Ethnopharmacology*. **39**: 129-139.

Dietz, D.D., Leininger, J.R., Rauckman, E.J., Thompson, M.B., Chapin, R.E:, Morrissey, R.L. and Linve, B.S. 1991. Toxicity studies of acetone administered in the drinking water of rodents. *Toxicological Sciences*. **17**: 347-346.

Eisenbrand, G., Pool-Zobel, B., Baker, V., Balls, M., Blaauboer, B.J., Boobis, A., Carere, A., Kevekordes, S., Lhuguenot, J.C., Pieters, R. and Kleiner, J. 2002. Methods of *in vitro* toxicology. *Food Chemical Toxicology*. **40**: 193-236.

Eloff, J.N. 1998.The presence of antibacterial compounds in *Anthocleista grandiflora. South African Journal of Botany*. **64**: 209-212.

Eloff, J.N. 1998b. Which extractant should be used for the screening and isolation of antimicrobial components from plants *Journal of Ethnopharmacology*. **160**: 1-8.

Eloff, J.N. 1998c. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plants extracts for bacteria. *Planta Medica*.**64**: 711-713.

Eloff, J.N. 1999b. The antibacterial activity of 27 Southern African members of the *Combretaceae*. *South African Journal of Science*. **95**: 148-152.

Eloff, J.N. 2000. A proposal on expressing the antibacterial activity of plant extracts-a small first step in applying scientific knowledge to rural primary health care in South Africa. *South African Journal of Science*. **96**: 116-118.

Eloff, J.N. 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) (Anarcardiaceae) bark and leaves. *Journal of Ethnopharmacology*. **76**: 305-308.

Eloff, J.N. 2004.Quantifiying the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine*. **11**: 370-371.

Eloff, J.N., Famakin, J.O. and Katerere, D.R.P. 2005. *Combretum woodii* (Combretaceae) leaf extracts have high activity against Gram-negative and Gram-positive bacteria. *African Journal of Biotechnology*. **4**: 1161-1166.

Eloff, J.N., Angeh, I. and McGaw, L. 2006. A plant antifungal product from *Melianthus comosus* (Melianthaceae) leaf extracts. GA Conference Helsinki September 2006. *Planta Medica*. **72**: 982.

Farnsworth, N.R. 1984.The role of medicinal plants in drug development, in *Natural products and Drug Development*. edited by P. Krogsgaard-Larsen & S.B. Christensen & H. Kofod. London: Balliere, Tindall and Cox, pp. 8-9.

Fokkema, E., Groen, H.J., Helder, M.N., De Vries, E.G. and Meijer, C. 2002. JM216-JM118-, and cisplastin-induced cytotoxicity in relation to platinum-DNA adduct formation, glutathione levels and p53

status in human tumour cell line with differential sensitivities to cisplatin. *Biochemical Pharmacology*. **63**: 1989-1996.

Frost and Sullivan .2005. Strategic Review of the European Ban of Antibiotic Growth Promoters in Animal Feed.Research and Markets: www.researchand markets.com.

Gelfand, M., Mavi, S., Drummond, R.B. and Ndemera, B. 1985. The Traditional Medical Practicioner in Zimbabwe. Gweru, Zimbabwe: Mambo Press.

Gundidza, M. 1986a. Screening of extracts from Zimbabwean higher plants II. Antifungal properties. *Fitoterapia***57**: 111-114.

Gundidza, M. 1986b. Activity of methanol extract from *Ziziphus mucronata* against *Candida albicans*. *Zimbabwe Science News*. **20**: 107-108.

Hagerman, A.E. 1986.Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology.***13**: 437-449.

Hagerman, A.E. 1998.*The Tannin Handbook*. http://www.users.muohio.edu/hagermae/tannin.pdf; 20.06.2008.

Harborne, S.B. and Baxter, H. 1995.Phytochemical Dictionary. A handbook of bioactive compounds from plants. London: Taylor and Francis.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K.E., Mshiu, E.N. and Samuelsson, G. 1982. Inventory of plants used in traditional medicine in Tanzania. 1. Plants of the families *Acanthaceae-Cucurbitaceae*. *Journal of Ethnopharmacology*. **6**: 29-60.

Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P.J. and Lee, C.C. 2007. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods*. **42**:377-387.

Hutchings A., Scott, A.H., Lewis, G. and Cunningham, A. 1996**.** Zulu Medicinal Plants, An inventory. Pietermaritzburg: University of Natal Press.

Itamochi, H., Kigawa, J., Sultana, H., Iba, T., Akeshima, R., Kamazawa, S., Kanamori, Y.and Terakawa, N. 2002. Sensitivity to anticancer agents and resistance mechanisms in clear cell carcinoma of the ovary. *Japanese Journal of Cancer Research*. **93**: 723-728.

Johnson, B.A. 1997. Market Report. *Herbal Gram*. **40**: 49-50.

Kalapos, M.P. 2003. On the mammalian acetone metabolism: from chemistry to clinical implications. *Biochimica et Biophysica Acta (BBA) – General Subjects*. **1621**: 122-139.

Khater, H.F. and Khater, D.F. 2009. The insecticidal activity of four medicinal plants against the blowfly *Lucilia sericata* (Diptera: Calliphoridae). *International Journal of Dermatology*. **48**: 492-497.

Kerharo, J. and Adam, J.G. 1974. La Pharmacopée Sénégalaise Traditionelle. Paris: Vigot.

Kòtze, M. and Eloff, J.N. 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (*Combretaceae*). *South African Journal of Botany.***68**: 62-67.

Lawton, J.R. & Rogers, C.B. 1993. Localisation of triterpenoids in young leaves of *Combretum molle*. *Electron Microscopic Society of Southern Africa.***21**: 27.

Lawton, J.R., Govender, H. and Rogers, C.B. 1991. Mollic Acid Glucoside: A Possible (Third World) Answer to the control of Schistosomiasis in South and Central Africa. *Planta Medica.***57** (suppl. 2): A74.

Liu, J., Sato, C. and Marumo, F. 1991. Characterization of the acetaminophen-glutathione conjugation reaction by liver microsomes: Species difference in the effects of acetone. *Toxicology Letters.* **56**: 260-274.

Liu, C. & Chen, Y., Cheng, M., Lee, S., Abd-el-Razek, M. H., Chang, W., Chen, Y. and Chen, I. 2008. Cytotoxic constituents from the root wood of Formosan *Michelia compressa*. *Journal of the Chilean Chemical Society.* **53**: 1523-1524.

Mabogo, D.E.N. 1990. The Ethnobotany of the Vhavenda. *Unpublished Master of Science Thesis*. University of Pretoria.

Mahato, S.B. and Kundu, A.P. 1994. 13CNMR spectra of pantacyclic triterpenoids – a compilation and some salient features. *Phytochemistry*. **37**: 1517-1575.

Mampane, K.J., Joubert, P.H. and Hay, I.T. 1987. *Jatropha curcas*: use as a traditional Tswana medicine and its role as a cause of acute poisoning. *Phytotherapeutic Research*. **1**: 50-51.

Masika, P.J. and Afolayan, A.J. 2002.Antimicrobial activity of some plants use for the treatment of livestock disease in the Eastern Cape, South Africa. *Journal of Ethnopharmacology*. **84**: 129-134.

Masoko, P., Picard, J. and Eloff, J.N. 2007. The antifungal activity of twenty-four Southern African *Combretum* species (Combretaceae). *South African Journal of Botany*. **73**: 173-183.

McGaw, L.J., Jäger, A.K. and Van Staden, J. 2002.. Variation in antibacterial activity of *Schotia* species. *South African Journal of Botany*. **68**: 41-46.

Mitchell, D., Wells, C., Hoch, N., Lind, K., Woods, S.C. and Mitchell, L.K. 1976. Poison induced pica in rats. *Physiology & Behaviour*. **17**: 691-697.

Mensah, A.Y., Sampson, J., Houghton, P.J., Hylands, P.J., Westbrook, J., Dunn, M., Hughes, M.A. and Cherry, G.W. 2001. Effects of *Buddleja globosa* leaf and its constitutents relevant to wound healing. *Journal of Ethnopharmacology*. **77**: 219-226.

Moldeus, P. and Gergely, V. 1980. Effect of acetone on the activation of acetaminophen. *Toxicologically. applied. Pharmacology.***53** :9-13.

Mosmann, T. 1983. Rapid colorimetric assay of cellular growth and survival:Application of proliferation and cytotoxicity assays. *Journal of Immunological Methods*. **95**: 55-63.

National Committee for Clinical Laboratory Standards, 1992. Performance Standards for Antimicrobial Susceptibility Testing; Fourth information supplement. Pennsylvania; USA: NCCLS M100-S4.

Nimri, L.F., Megdam, M.M. and ALKOFAHI, A. 1999.Antibacterial activity of Jordanian medicinal plants. *Pharmaceutical Biology.***37**: 196-201.

NTP. 1991. Toxicity studies of acetone in F344/N rats and B6C3F_1 mice (drinking water studies). Research Triangle Park, North Carolina, US Department of Health and Human Services, National Toxicology Program, 38 pp (Technical Report NO. 3; NIH Publication NO. 91-3122).

Oberem, P., Odendaal, D., Oberem, P.T., Snyman, M.G.S., Ludwig, L. and Mynhardt, H. 2006. Diseases and Parasites of Cattle, Sheep and Goats in South Africa. Pretoria: AFRIVET BUSINESS MANAGEMENT.

OECD/OCDE, adopted 17th of December 2001. OECD guideline for testing of chemicals. Acute oral toxicity – Acute toxic class method. www.oecd.org.

Pegel, K.H. and Rogers, C.B. 1976. Mollic acid 3-β-D-glucoside, A novel 1α-hydroxycycloartane saponin from *Combretum molle* (*Combretaceae*). *Tetrahedon Letters.***47**: 4299-4302.

Pegel, K.H. and Rogers, C.B. 1985. The characterization of mollic acid 3β-D-xyloside and its genuine aglycone mollic acid, two novelα-hydroxycycloartenoids from *Combretum molle*. *Journal of the Chemical Society Perkins Transactions.* **1**:1711-1715.

Plaa, G.L. and Traiger, G.J. 1972; Mechanism of potentiation of CCl4-induced hepatotoxicity. In: Loomis TA ed. Pharmacology and the future of man – Volume 3: Toxicological problems. Basel, Switzerland, Larger, pp 100-113.

Repetto, G., Delpeso, A. and Zurita, J.L. 2008. Neutral red assay for the estimation of cell viability/cytotoxicity. *Nature Protocol*. **3**: 1125-1131.

Riipi, M., Ossipov, V., Lempa, K., Haukioja, E., Koricheva, J., Ossipova, S. and Pihlaja, K. 2002. Seasonal changes in birch leaf chemistry: are there trade-offs between leaf growth and accumulation of phenolics. *Oecologia*. **130**: 380-390.

Rogers, C.B. and Thevan, I. 1986. Identification of Mollic acid α-L-arabinose, a 1α-hydroxycycloartenoid from *Combretum molle* leaves. *Phytochemistry*. **25**: 1759-1761.

Saxena, V.K. and Shrma, R.N. 1999.Antimicrobial activity of essential oil of *Lantana aculeate*. *Fitoterapia*. **70**: 59-60.

Schilcher, H., Kammerer, S. and Wegener, T. 2007. *Leitfaden Phytotherapie* (3. Auflage). München: Elsevier GmbH.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bodesch, H., Kenney, S. and Boyd, M.R. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*. **82**: 1107-1112.

Smith, A. 1895. A contribution to South African Materia Medica. 3rd edition. Lovedale: Lovedale Press.

Stahl, E. (edit.), 1969. Thin Layer Chromatography. A Laboratory Handbook. 2nd edition. Berlin, Heidelberg, New York: Springer-Verlag.

Stecher, G., Huck, C.W., Stöggl, W.M. and Bonn, G.K. 2003. Phytoanalysis: a challenge in phytomics. *Trends in Analytical Chemistry*. **22**: 1-14.

Suffness, M. and Douros, J. 1979. Drugs of Plant Origin. Methods in cancer research. **26**: 73-126.

Tscheche R., Elgamal, M., Eckardt, G. and Von Radloff, M. 1974. Peptide alkaloids from *Ziziphus mucronata*. *Phytochemistry* (Oxford). **13**: 2328.

Van Wyk, B.E., Van Oudtshoorn, B. and Gericke, N. 1997. Medicinal plants of South Africa. Pretoria: Briza Publications.

Van Wyk, B. and Van Wyk, P. 1997. Field guide to trees of southern Africa. Cape Town: Struik Publishers.

Voigt, W. 2005. Sulphorhodamine B assay and chemosensitivity. *Methodology of Molecular Medicine*. **110**: 39-48.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of Southern and Eastern Africa.2nd edition. Livingstone, London.

Williamson, E., 2001. Synergy and other interactions in phytomedicines. *Phytomedicine*. **8**: 401-409.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora environment. *International Journal of Antimicrobial Agents*. **14**: 321-325.

WHO: WORLD HEALTH ORGANIZATION 1979. The selection of essential drugs. *Second report of the WHO Expert Committee. WHO Technical Report Series*. **641**: 1-44.

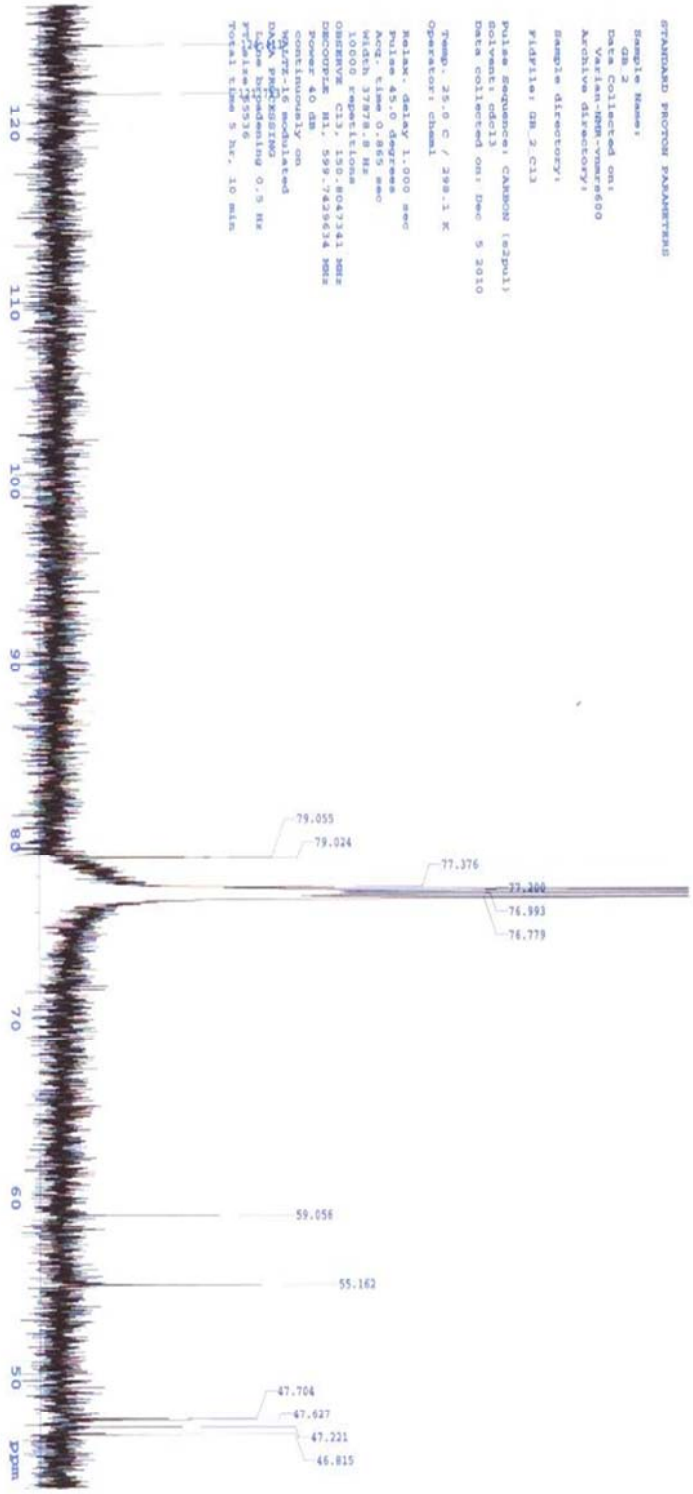
WHO: WORLD HEALTH ORGANIZATION, EHC, 207.1998: Environmental Health Criteria 207 Acetone. Geneva. 1998. http://www.inchem.org/documents/ehc/ehc/ehc207.htm

Wynn, S.G. and Fougère, B.J. 2007. Veterinary Herbal Medicine. USA: Mosby Elsevier.

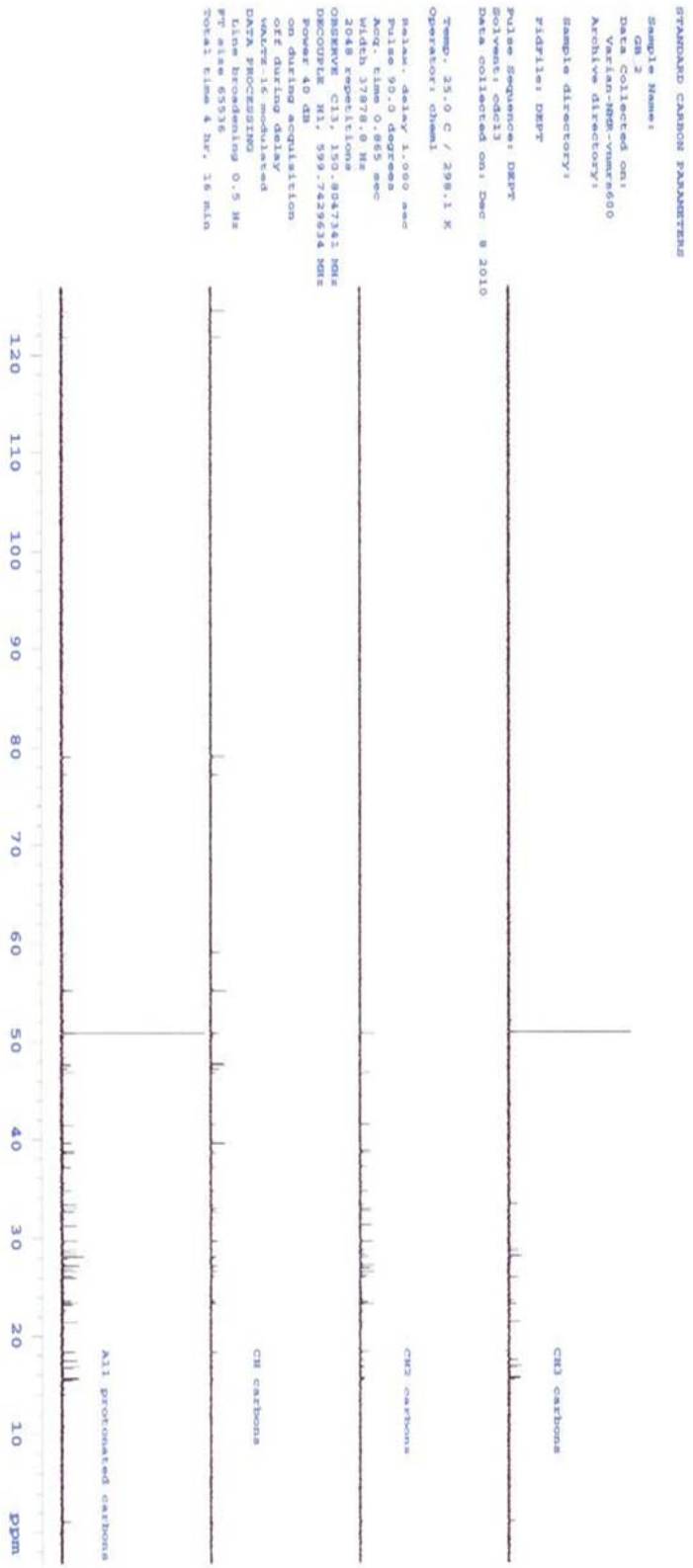
Appendix A

A 1: ^{1}H-NMR Spectroscopy of C1

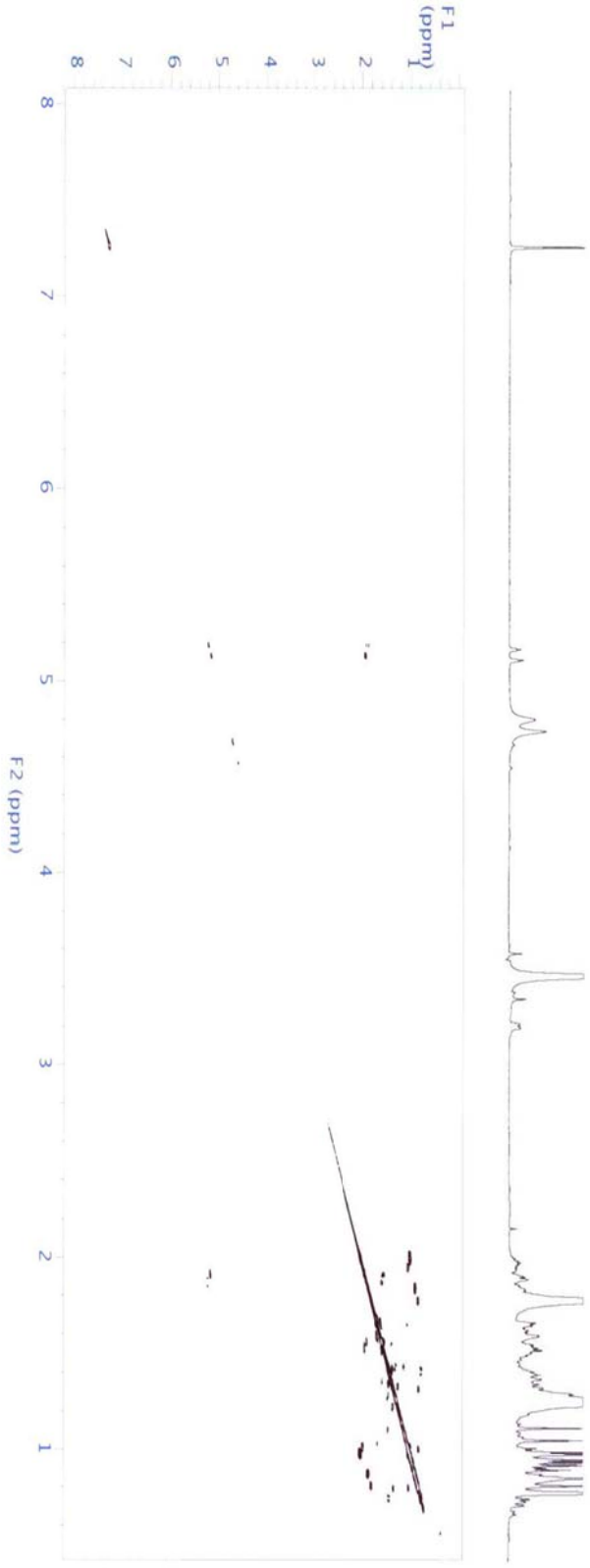
A 2: ^{13}C-NMR Spectroscopy of C1

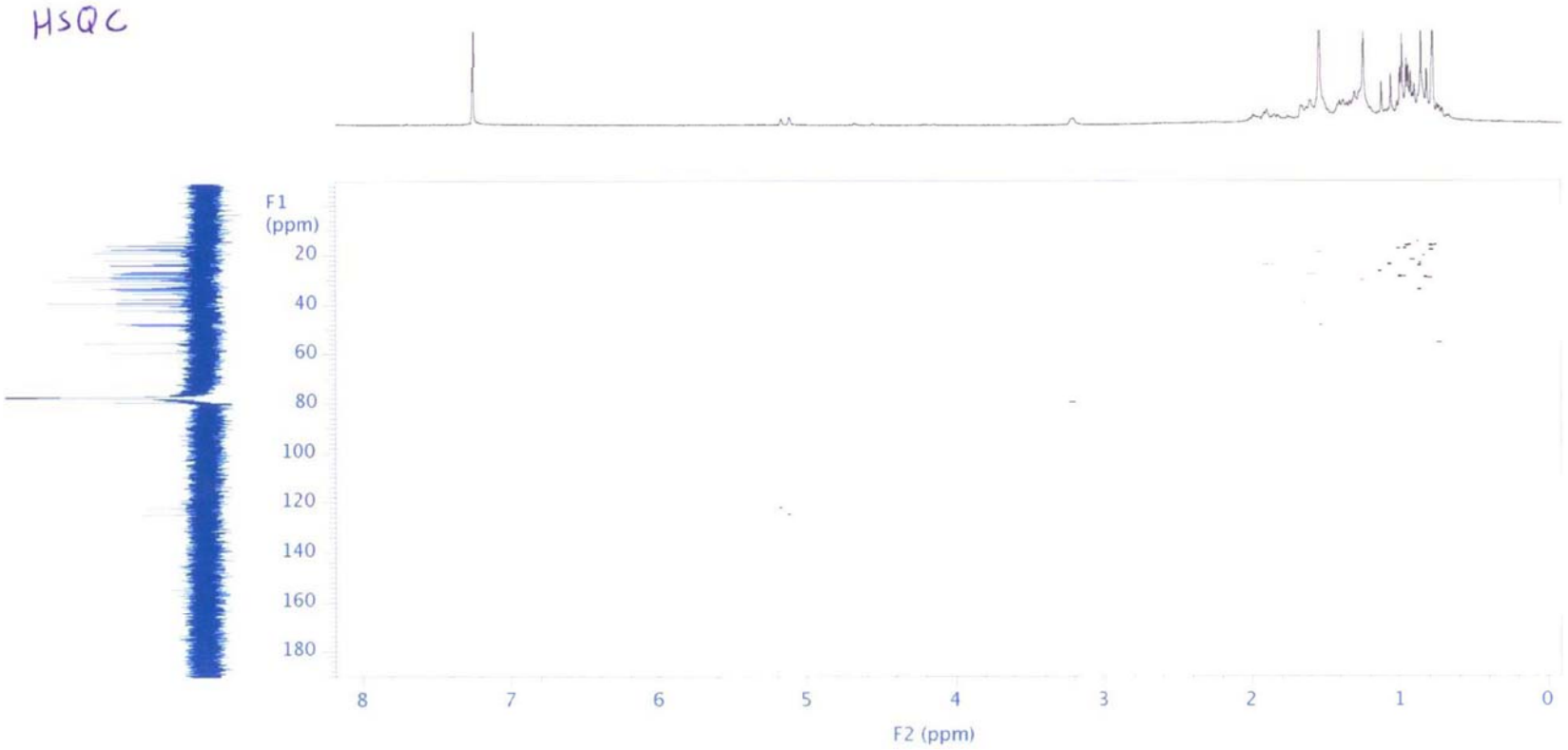


A 3: ^{13}C-DEPT-NMR Spectroscopy of C1

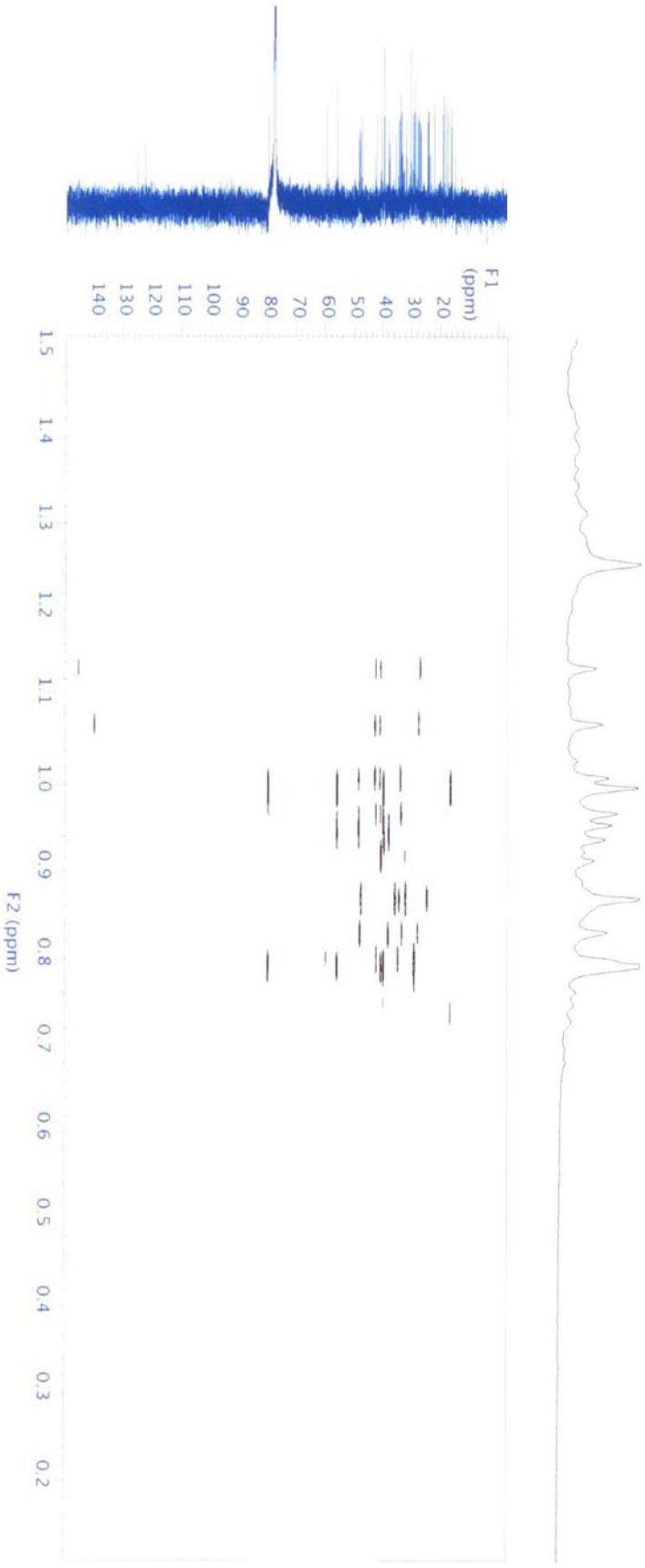


A 4: COSY-NMR Spectroscopy of C1





A 5: HSQC-NMR Spectroscopy of C1


F1 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
F2 (ppm)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2

Appendix B

Table B 1: Dosing regimen, clinical signs and macroscopic lesions and percentage of weight gain or loss in animals dosed with *Deinbollia oblongifolia*

Rat	Dosing quantity	Clinical signs	Macroscopic lesions	% (+, -)
1	5 mg/kg	Scratching; eye discharge after dosing; pulmonary distress from 2 hours after dosing; gasping blood on day 2; warfarin spots; euthanized day2	Moderate gas accumulation in the stomach; Moderate hepatic centrolobular congestion and blood pooling with dilated centrilobular sinousoids; spleen with extramedullary haemopoiesis in moderately congested red pulp; mild lung congestion with mild protein rich lung oedema and focal areas of atelectasis and emphysema; mild haemothorax	NA
24	5 mg/kg	Frantically eating paper; depression; unsteady gait; scratching right after dosing; piloerection; pulmonary distress after dosing on day 2; euthanized day 3	Mild adrenal medullary congestion; mild autolysis in the large and small intestine; pulmonary congestion of the alveolar walls; active white pulp in spleen	-1.5%
28	5 mg/kg	Depression; after dosing on day 2 slight pulmonary distress; euthanized day 3	Mild bilateral medullary congestion and sinusoidal dilatation of the adrenal; mild liver congestion; mild pulmonary congestion; mild splenomegaly with moderate congestion in splenic red pulp	
29	5 mg/kg	Scratching; after dosing on day 2 pulmonary distress; piloerection, unsteady gait from day 2 on; euthanized day 3	Mild autolytic changes in uterine horn; mild pulmonary congestion; active normal white pulp and mild extramedullary haemopoiesis; mild haemothorax	-4.2%
33	5 mg/kg	None; euthanized day 3	Minimal autolysis in small intestine; mild pulmonary congestion and oedema; splenic white pulp is active hyperplastic while mild extramedullary haemopoiesis within the splenic red pulp; mild haemothorax	-0.2%
36	5 mg/kg	Piloerection; after dose on day 2 pulmonary distress; euthanized day 3	Mucometra; pulmonary congestion and minimal oedema; mild haemothorax	-12.8%
3	20 mg/kg	Pica; after dose day 2 swollen abdomen; euthanized day 3	Minimal autolytic changes in intestinal mucosa; mild lung atelectasis; prominent splenic white pulp and	+0.4%

			moderate extramedullary haemopoiesis in splenic red pulp; severe haemothorax	
9	20 mg/kg	Scratching; depression; piloerection; nasal discharge; pulmonary distress; terminated after four hours	Moderate gas accumulation in stomach and small intestine; mild congestion in adrenal medulla; mild multifocal tubular dilatation of collecting ducts in both kidney sections; pale lung; mild lung oedema and foci of atelectasis and emphysema; mild focal autolysis in pancreas; mild extramedullary haemopoiesis in the spleen with normal splenic red pulp and with pulp and mild haemosiderosis; mild congestion of the thymic medulla; small amount of blood in pericardium and in the thorax	NA
18	20 mg/kg	Scratching; after dose on day 2 swollen abdomen detected and pulmonary distress; euthanized day 3	Pulmonary congestion; moderate extramedullary haemopoiesis in splenic red pulp; moderate haemothorax	+4-5%
23	20 mg/kg	Depression; scratching; after dose on day 2 pica; piloerection on day 2; euthanized day 3	Mild autolytic changes in the small intestine; mild pulmonary congestion; mild extramedullary haemopoiesis in spleen; moderate haemothorax	+3.0%
25	20 mg/kg	None; euthanized day 3	Moderated congested adrenal medulla; moderate autolysis in intestinal mucosa of large intestine; moderate autolysis in the intestinal mucosa in the small intestine; ; moderate autolytic foci in pancreas; mild extramedullary haemopoiesis in splenic red pulp; moderate haemothorax	+2.6%
35	20 mg/kg	Pulmonary distress; unsteady gait; depression; piloerection; euthanized after 5 hours	Minimal gas accumulation in stomach; adrenal congestion; moderate congestion of the leptomeninges and neuroparenchyma; mild autolysis in small intestine; congestion in liver cortex; moderated accentuated lobulation in liver with moderated centrilobular blood pooling; moderate lung congestion with lung oedema and multifocal areas of atelectasis; mild foci of autolysis in pancreas; moderate extramedullary haemopoiesis in the splenic red pulp; mild haemothorax	NA
2	50 mg/kg	Scratching; piloerection day 2; euthanized day 3	Gas accumulation in the stomach; mild congestion in adrenal medulla; minimal autolytic changes in small	+3.5%

			intestine; mild cortical congestion in kidney; mild hepatic congestion in the central part of the hepatic nodules and mild accentuated lobulation; mild pulmonary congestion; moderate extramedullary haemopoiesis in spleen; unilateral anophtalmia from birth	
5	50 mg/kg	Scratching; piloerection; depression; warfarin spots on day 2 as well as swollen abdomen; after dosing on day 2 pulmonary distress and unsteady gait; euthanized day 3	Mild renal tubular dilatation; moderate hepatic congestion and blood pooling; focal autolytic changes in pancreas; moderate hyperplasia of the lymphoid tissue in the splenic white pulp and extramedullary haemopoiesis is moderate in the red pulp; mild congestion of the thymic tissue; internal congestion and splenomegaly; severe haemopericardium	-1.7%
11	50 mg/kg	Scratching; depression; piloerection; unsteady gait; pulmonary distress; euthanized day 3	Mild pulmonary oedema and congestion; minimal pancreatic autolysis; active white pulp and moderate congestion in red pulp	-1.5%
12	50 mg/kg	Depression; unsteady gait; piloerection; scratching; pulmonary distress; after dose on day 2 severe depression; euthanized day 3	Mild hepatic congestion in the portal areas; mild lung oedema and areas of atelectasis; moderate extramedullary haemopoiesis in the splenic red pulp; mild splenic congestion	-2.6%
26	50 mg/kg	Depression; scratching; euthanized day 3	Mild sinusoidal dilatation and congestion of the adrenal medulla; mild portal and parenchymal foci of lymphocytic infiltrations in liver; mild lung oedema; moderate extramedullary haemopoiesis in spleen; mild autolysis in stomach; mild haemopericardium and haemothorax	+1.3%
38	50 mg/kg	Scratching; dose on day 2 struggle to dose; bloated abdomen on day 2; euthanized day 3	Minimal mucosal autolysis in small intestine; mild sinusoidal blood pooling in liver; foci of autolysis in pancreas; mild extramedullary haemopoiesis in the red pulp of the spleen	-1.2%

Table B 2: Clinical chemistry parameters of rats dosed with 5 mg/kg of the crude extract of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 1	Rat 24	Rat 28	Rat 29	Rat33	Rat 36
TSP (g/l)	58.5 (± 2.3)	55	55.3	53	53.1	53.4	59
ALB (g/l)	30.8 (± 1.1)	39.6	35.7	37.4	35.7	31.3	41.9
GLOB (g/l)	31 - 33[b]	15.4	19.6	15.6	17.4	22.1	17.1
A/G	0.95 – 0.96[c]	2.59	1.82	2.4	2.06	1.42	2.44
ALT (U/l)	57.5 (±22.5)	31	75	71	45	55	30
ALP (U/l)	290 (± 63)	94	132	114	93	146	108
AST (U/l)	78.1 (± 13.0)	92	191	139	88	118	109
GGT (U/l)	5 – 6[b]	5	2	0	1	0	0
Urea (mmol/l)	9.46 (± 0.84)	4.2	6.7	7.7	9.3	7.7	7.9
Crea (µmol/l)	47.6 (± 7.4)	33	30	31	35	23	37
Na	145 (± 5)	136	139.6	141.5	140.5	139.2	137.6
K	4.95 (± 0.65)	3.59	4.57	4.01	4.31	4.1	3.99
Ca	2.4 (± 0.2)	2.47	2.67	2.75	2.52	2.57	2.52

Table B 3: Clinical chemistry parameters of rats dosed with 20 mg/kg of the crude extract of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 3	Rat 9	Rat 18	Rat 23	Rat25	Rat 35
TSP (g/l)	58.5 (± 2.3)	46.9	ND	53.8	56.2	55.4	ND
ALB (g/l)	30.8 (± 1.1)	10.8	ND	40	40.4	40.3	ND
GLOB (g/l)	31 - 33[b]	36.1	ND	13.8	15.8	15.1	ND
A/G	0.95 – 0.96[c]	0.3	ND	2.89	2.56	2.66	ND
ALT (U/l)	57.5 (±22.5)	52	ND	65	86	68	ND
ALP (U/l)	290 (± 63)	104	ND	127	175	151	ND
AST (U/l)	78.1 (± 13.0)	218	ND	168	166	157	ND
GGT (U/l)	5 – 6[b]	7	ND	1	1	1	ND
Urea (mmol/l)	9.46 (± 0.84)	5	ND	8.5	8.3	8.1	ND
Crea (µmol/l)	47.6 (± 7.4)	22	ND	35	31	28	ND
Na	145 (± 5)	130.7	ND	139.4	142.7	141.1	ND
K	4.95 (± 0.65)	5.44	ND	5.27	4.66	4.99	ND
Ca	2.4 (± 0.2)	2.11	ND	2.93	2.72	2.86	ND

Table B 4: Clinical chemistry parameters of rats dosed with 50 mg/kg of the crude extract of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 2	Rat 5	Rat 11	Rat 12	Rat26	Rat 38
TSP (g/l)	58.5 (± 2.3)	56.7	52.8	56.8	57.1	56.1	53.9
ALB (g/l)	30.8 (± 1.1)	42.1	35.3	42.1	43.5	41.7	40
GLOB (g/l)	31 - 33[b]	14.6	17.5	14.7	13.6	14.4	13.9
A/G	0.95 – 0.96[c]	2.88	2.02	2.85	3.18	2.9	2.87
ALT (U/l)	57.5 (±22.5)	67	53	57	65	71	65
ALP (U/l)	290 (± 63)	170	106	177	158	165	137
AST (U/l)	78.1 (± 13.0)	115	179	214	212	136	123
GGT (U/l)	5 – 6[b]	2	2	3	3	1	0
Urea (mmol/l)	9.46 (± 0.84)	9	10	6.1	10.9	6.3	7.3
Crea (µmol/l)	47.6 (± 7.4)	28	36	36	34	27	29
Na	145 (± 5)	140.5	140.9	142.4	141.1	141.2	141.7
K	4.95 (± 0.65)	4.17	5.09	4.92	5.07	4.21	3.9
Ca	2.4 (± 0.2)	2.68	2.66	3.08	2.71	2.63	2.62

Table B 5: Dosing regimen, clinical signs and macroscopic lesions and percentage of weight gain or loss in animals dosed with the chloroform fraction of *Deinbollia oblongifolia*

Rat	Dosing quantity	Clinical signs	Macroscopic lesions	% (+, -)
4	50 mg/kg	Scratching; euthanized day 3	Moderate cortical congestion bilateral in adrenal glands; mild autolytic changes in the intestinal mucosa of small intestine; mild cortical congestion in kidney; mild hepatic congestion; focal lung congestion; moderated extramedullary haemopoiesis in the red pulp of the spleen with moderate haemosiderosis; moderate haemorrhages in the heart and mild haemopericardium	-1.5%
13	50 mg/kg	Scratching; depression; pulmonary distress; unsteady gait; piloerection; warfarin spots on day 2 and gasping blood; euthanized after 21 hours	Severe gas accumulation in the stomach; mild autolytic changes in the intestinal mucosa; mild congestion in both Kidneys; moderate centrilobular congestion with sinusoidal dilatation in liver; protein-rich lung oedema and focal congestion; mild extramedullary haemopoiesis and haemosiderosis of the red splenic pulp and active lymphoid tissue in the splenic white pulp	NA
16	50 mg/kg	Pulmonary distress; euthanized	Small amount of gas in small intestine; mild medullary	NA

		after four hours	congestion in adrenal; mild congestion in leptomeninges and neuroparenchyma; moderate dilatation of uterus and few lymphocytic cells in the endometrial lamina propria; moderate mucometra; mild congestion of mucosa of the small intestine; centrilobular blood pooling in liver with mild to moderate accentuated liver lobulation; focal atelectasis in the lung; congestion of red splenic pulp	
19	50 mg/kg	Pica; swollen abdomen on day 2; euthanized day 3	Minimal congestion in the adrenal medulla; mild autolysis in large and small intestine; in the renal crest, small foci of lymphocytic infiltrations in one of the kidney sections; mild atelectasis and lung congestion; mild autolytic changes in pancreas; congestion of red splenic pulp	+2.1%
31	50 mg/kg	Pulmonary distress after dosing on day 2; warfarin spots day 2; piloerection day 3; euthanized day 3	Mild mucosal autolysis in small intestine; mild congestion of the cortico –medullary junction; mild atelectasis in one lung section; moderate extramedullary haemopoiesis and mild splenic congestion	+3.3%
39	50 mg/kg	Unsteady gait; piloerection, depression, unsteady gait and pulmonary distress day 2; euthanized day3	Mild autolytic changes in small intestine; mild pulmonary congestion; mild extramedullary haemopoiesis in spleen; mild haemopericardium and haemothorax	+4.4%
7	125 mg/kg	Pulmonary distress; depression; nasal discharge; piloerection; pica; depression; euthanized day 3	Mild autolysis in the small intestine; cortical congestion in both kidneys; bilateral pulmonary atelectasis and lung congestion; moderate extramedullary haemopoiesis and active lymphoid tissue; moderate haemothorax	-11.4%
8	125 mg/kg	Depression; blood on nose after dose on day 2; piloerection; pulmonary distress day 2; unsteady gait day 2; euthanized day 3	Small amount of gas in stomach; mild autolytic changes in small intestine; mild renal cortical congestion in both kidneys; mild atelectasis in the lung; mild autolytic changes in spleen; moderate congestive splenomegaly and active lymphoid tissue in the splenic white pulp	-9.0%

10	125 mg/kg	Depression; scratching; pulmonary distress after dose on day2; euthanized day 3	Congested adrenal cortices; mild autolysis in large and small intestine; mild pulmonary atelectasis; mild autolysis in pancreas; active splenic white pulp and mild extramedullary haemopoitic cells in red pulp; mild haemopericardium	+3.7%
14	125 mg/kg	Scratching on day2; depression; piloerection, unsteady gait; pulmonary distress day2; euthanized day 3	Mild cortical congestion in adrenal; mild autolytic changes in small intestine; mild hepatic congestion and sinusoidal blood pooling; moderate pulmonary congestion; mild autolytic changes in pancreas; moderated extramedullary haemopoiesis in the splenic red pulp	+4.5%
17	125 mg/kg	Depression; blood after dosing on day2; pulmonary distress on day2; piloerection; euthanized day 3	Mild adrenal cortical congestion; mild leptomeningeal congestion; mild autolysis in large intestine and small intestinal mucosa; mild pulmonary congestion; active white pulp and moderate extramedullary haemopoiesis in the splenic red pulp; haemothorax and haemopericardium	+1.7%
20	125 mg/kg	Pica; scratching; pulmonary distress on day2; euthanized day 3	Mild adrenal congestion; mild atelectasis and congestion of the anterior lobe of the lung; mild extramedullary haemopoiesis and mild haemosiderosis; mild haemothorax	+1.7%
22	300 mg/kg	Scratching; depression; piloerection; warfarin spots on eyes on day 2; piloerection; pulmonary distress; euthanized day3	Moderate autolytic changes in the small intestine; pulmonary congested and show atelectasis; mild autolysis in pancreas; red splenic pulp with moderate extrtamedullary haemopoiesis; moderate haemothorax	-2.1%
30	300 mg/kg	Unsteady gait; scratching; depression; piloerection; pulmonary distress after dosing on day2; euthanized day3	Mild pulmonary congestion; mild congestion of the red splenic pulp	-1.4%

37	300 mg/kg	Depression; piloerection; euthanized day 3	Adrenal cortex and medulla congested; mild pulmonary congestion with foci atelectasis; prominent and active splenic lymphoid tissue; mild extramedullary haemopoiesis in the red splenic pulp; mild haemothorax	-0.1%
40	300 mg/kg	Depression, piloerection; warfarin spots; pulmonary distress day 2; euthanized day 3	Mild gas accumulation in stomach; mild congestion of the deeper cortical layer and adrenal medulla; mild hepatic centrilobular congestion; moderated atelectasis and pulmonary congestion; mild autolytic changes in pancreas; active splenic white pulp	-0.3%
41	300 mg/kg	Depression; pulmonary distress; piloerection; unsteady gait after dose on day2; euthanized day 3	Mild autolysis in large and small intestine; minimal pulmonary congestion; mild autolytic changes in the pancreas; active splenic white pulp	+1.0%
42	300 mg/kg	Unsteady gait; depression; drinking a lot; piloerection; unsteady gait and warfarin spots on eyes as well as swollen abdomen on day 2; pulmonary distress day 2; euthanized day 3	Mild gas accumulation in stomach; mild blood pooling and congestion in liver; mild pulmonary congestion and atelectasis; moderated extramedullary haemopoiesis in the splenic red pulp; well populated lymphocytes in thymus; moderate haemothorax	-3.2%

Table B 6: Clinical chemistry parameters of rats dosed with 50 mg/kg of the chloroform fraction of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 4	Rat 13	Rat 16	Rat 19	Rat31	Rat 39
TSP (g/l)	58.5 (± 2.3)	53.2	51.7	ND	54.9	56.1	55.6
ALB (g/l)	30.8 (± 1.1)	36.3	26.9	ND	42.1	41.6	39.2
GLOB (g/l)	31 - 33[b]	16.9	24.8	ND	12.8	14.5	16.4
A/G	0.95 – 0.96[c]	2.15	1.09	ND	3.3	2.86	2.38
ALT (U/l)	57.5 (±22.5)	58	93	ND	67	67	60
ALP (U/l)	290 (± 63)	124	64	ND	137	157	163
AST (U/l)	78.1 (± 13.0)	159	420	ND	150	88	90
GGT (U/l)	5 – 6[b]	4	19	ND	2	< 0	0
Urea (mmol/l)	9.46 (± 0.84)	7.1	3.8	ND	6.6	7.8	8.3
Crea (µmol/l)	47.6 (± 7.4)	35	< 18	ND	32	29	29
Na	145 (± 5)	140.1	113.1	ND	141.7	141.8	139.7
K	4.95 (± 0.65)	3.93	ND	ND	4.38	3.85	4.8
Ca	2.4 (± 0.2)	2.58	1.85	ND	2.61	2.7	2.68

Table B 7: Clinical chemistry parameters of rats dosed with 125 mg/kg of the chloroform fraction of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 7	Rat 8	Rat 10	Rat 14	Rat17	Rat 20
TSP (g/l)	58.5 (± 2.3)	49.4	48.7	52.4	55.9	52.4	50.7
ALB (g/l)	30.8 (± 1.1)	23.1	36.1	35.9	39.7	40.3	37.3
GLOB (g/l)	31 - 33[b]	26.3	12.6	16.5	16.2	12.1	13.4
A/G	0.95 – 0.96[c]	0.88	2.86	2.19	2.45	3.34	2.77
ALT (U/l)	57.5 (±22.5)	69	44	78	63	55	46
ALP (U/l)	290 (± 63)	62	111	142	139	138	138
AST (U/l)	78.1 (± 13.0)	250	155	100	156	97	132
GGT (U/l)	5 – 6[b]	7	2	3	1	0	1
Urea (mmol/l)	9.46 (± 0.84)	10.8	10.3	6.9	9.1	7.4	9.4
Crea (µmol/l)	47.6 (± 7.4)	40	35	28	25	31	30
Na	145 (± 5)	135.1	139.4	140.5	140.9	141.9	143.2
K	4.95 (± 0.65)	5.73	4.64	4.16	2.16	4.52	4.08
Ca	2.4 (± 0.2)	2.55	2.49	2.55	2.6	2.68	2.6

Table B 8: Clinical chemistry parameters of rats dosed with 300 mg/kg of the chloroform fraction of *Deinbollia oblongifolia*

Analytes	Reference values	Rat 22	Rat 30	Rat 37	Rat 40	Rat41	Rat 42
TSP (g/l)	58.5 (± 2.3)	56.8	54.7	54.4	54.5	53.1	49
ALB (g/l)	30.8 (± 1.1)	41.7	40.8	41.6	34.6	38	23
GLOB (g/l)	31 - 33[b]	15.1	13.9	12.8	19.9	15.1	26
A/G	0.95 – 0.96[c]	2.77	2.93	3.25	1.74	2.53	0.89
ALT (U/l)	57.5 (±22.5)	69	66	73	80	67	55
ALP (U/l)	290 (± 63)	134	135	148	130	142	127
AST (U/l)	78.1 (± 13.0)	108	147	98	252	134	86
GGT (U/l)	5 – 6[b]	1	1	< 0	3	< 0	< 0
Urea (mmol/l)	9.46 (± 0.84)	7.7	9.5	7.5	6.1	7	8.1
Crea (µmol/l)	47.6 (± 7.4)	33	35	31	30	26	31
Na	145 (± 5)	140.8	137.6	139.9	138.6	139.5	136.9
K	4.95 (± 0.65)	4.37	4.75	4.5	5.2	3.89	4.01
Ca	2.4 (± 0.2)	2.82	2.68	2.67	2.62	2.54	2.32

Table B 9: Dosing regimen, clinical signs and macroscopic lesions and percentage of weight gain or loss in animals dosed with the solvent control acetone

Rat	Dosing quantity	Clinical signs	Macroscopic lesions	% (+, -)
6	0 mg/kg	Pulmonary distress; piloerection; depression; euthanized after 9 hours	Moderated gas accumulation in stomach and small intestine; mild leptomeningeal congestion in brain; prominent apoptosis and moderated centrilobular blood pooling and accentuated lobulationin the liver; moderate diffuse protein-rich lung oedema and atelectasis and haemorrhage in the lung; mild lung congestion; moderate haemosiderosis and extramedullary haemopoiesis; severe haemothorax	NA
15	0 mg/kg	Depression; intraperitoneal dosing day 2 then unsteady gait, piloerection, heart palpitations; swollen abdomen; pulmonary distress; euthanized day 3	Mild medullary congestion in kidney; mild pulmonary congestion; moderate extramedullary haemopoiesis and normal splenic white pulp; mild haemothorax and haemopericardium	+0.1%

21	0 mg/kg	Dose on day 2 intraperitoneally; depression; pica; unsteady gait; piloerection; scratching; day 3 slight tendency to the left; euthanized day 3	Mild acute peritoneal inflammation in the mesentery of the small intestine; focal atelectasis and mild congestion in lung; active lymphoid tissue in the splenic white pulp with mild extramedullary haemopoiesis of the red pulp; mild thymus congestion, but thymic lymphoid tissue normal; mild haemothorax	-7.8%
27	0 mg/kg	Slight depression; died during the night	Stomach severely dilated with gas; segmental gas accumulation in small intestine; moderated congestion of the leptomeninges in brain; moderate autolysis in small and large intestine; moderated congestion of renal cortex; moderate to severe centrilobular blood pooling in liver; moderated pulmonary congestion and moderate lung oedema with protein-rich fluid in the alveoli; foci of atelectasis and emphysema; foci of autolytic changes in pancreas; moderate haemosiderosis in splenic red pulp; mild thymus congestion	NA
32	0 mg/kg	Depression; intraabdominal dosing day 2; depression; unsteady gait; piloerection; euthanized day 3	Mild congestion of the medullary adrenal; mild inflammation in the peri-adrenal fat; mild peritoneal mesenteric macrophage-rich reaction; moderate autolysis in the mucosa and neutrophils and macrophages in the mesentery; foci of autolysis and few macrophages on the peritoneal surface of pancreas; mild extramedullary haemopoiesis and moderate hamosiderosis in spleen	-7.5%
34	0 mg/kg	Warfarin spots; nasal discharge; depression; pulmonary distress; unsteady gait; oedema at eye; euthanized after 4 hours	Mild gas accumulation in stomach; mild dilatation of uterus associated with mucometra; mild kidney congestion; blood pooling and accentuated lobulation in liver; mild atelectasis and congestion in lung; active white pulp in spleen; mild splenic red pulp haemosiderosis; severe haemothorax	NA

Table B 10: Clinical chemistry parameters of rats dosed with the pure solvent (Acetone)

Analytes	Reference values	Rat 6	Rat 15	Rat 21	Rat 27	Rat32	Rat 34
TSP (g/l)	58.5 (± 2.3)	ND	45.7	50.4	ND	50	ND
ALB (g/l)	30.8 (± 1.1)	ND	26.2	32	ND	24.7	ND
GLOB (g/l)	31 - 33[b]	ND	19.5	18.4	ND	25.3	ND
A/G	0.95 – 0.96[c]	ND	1.35	1.74	ND	0.98	ND
ALT (U/l)	57.5 (±22.5)	ND	151	163	ND	157	ND
ALP (U/l)	290 (± 63)	ND	103	104	ND	113	ND
AST (U/l)	78.1 (± 13.0)	ND	478	410	ND	444	ND
GGT (U/l)	5 – 6[b]	ND	3	1	ND	5	ND
Urea (mmol/l)	9.46 (± 0.84)	ND	20.3	9.4	ND	6.1	ND
Crea (µmol/l)	47.6 (± 7.4)	ND	32	33	ND	26	ND
Na	145 (± 5)	ND	130.8	134.3	ND	132	ND
K	4.95 (± 0.65)	ND	4.52	5.57	ND	5.49	ND
Ca	2.4 (± 0.2)	ND	2.3	2.41	ND	2.34	ND