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Hypoglycaemic and antidiabetic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan-induced diabetic rats

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Diabetes is a chronic metabolic disorder characterized by dysregulation in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action. Recent studies have provided unequivocal evidence for the crucial role of prolonged hyperglycaemia in the development of chronic diabetic complications. *Myristica fragrans* Houttuyn (Myristicaceae) commonly known as nutmeg, used for both culinary and medicinal purposes in many countries. Since no scientific studies have been carried out on the seeds, this study was designed to investigate hypoglycaemic and antidiabetic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan-induced diabetic rats. The petroleum ether (60–80°C) extract of *Myristica fragrans* (PEMF) was administered orally in normal fasted, glucose fed (1.5 g/kg, p.o.) and alloxan (120 mg/kg, s.c.)-induced diabetic rats (n = 5). The blood glucose levels were estimated using glucometer (One Touch, Johnson and Johnson). In addition, changes in body weight, organ (liver, kidney and pancreas) weight, serum lipid profile and blood parameters (haemoglobin, erythrocytes and differential leukocytes) were assessed after two weeks in the extract treated diabetic rats (Table 1). We found that, oral pre-treatment with PEMF at dose of 200 mg/kg induced a significant ($P < 0.05$) decrease in blood glucose level i) from 56.5 ± 3.19 (0 h) to 49.75 ± 2.05 mg% (4 h) in normoglycaemic rats ii) from 145.75 ± 9.65 to 81.5 ± 4.03 mg% in oral glucose tolerance test (OGTT) at h compared to control glucose fed rats iii) from 326.25 ± 7.05 to 268.0 ± 9.6 mg% in alloxan- induced diabetic rats after daily treatment of PEMF for two weeks. After two weeks daily administration of PEMF, diabetic treated rats showed improvement in body weight, organ (liver and pancreas) weight, lipid profiles and haemoglobin content as compared to diabetic control rats. Thus, the present data indicates that *Myristica fragrans* possesses potential as an antidiabetic and warrants the need for further studies to elucidate its mode of action.

Table 1 Effect of two weeks treatment of PEMF on different blood parameters in alloxan-induced diabetic rats

Sr. No	Parameter	Normal control	Diabetic control	Diabetic + PEMF (200 mg/kg/day)	Diabetic + glibenclamide (0.40 mg/kg/day)
1	Body weight	228.75 ± 6.57	190 ± 7.07	214.25 ± 4.26*	215 ± 2.88*
2	Organ weight				
	Liver	6.01 ± 0.17	4.85 ± 0.38	5.89 ± 0.22*	5.90 ± 0.30
	Kidney	0.62 ± 0.04	0.54 ± 0.02	0.58 ± 0.02	0.59 ± 0.02
	Pancreas	0.42 ± 0.07	0.68 ± 0.03	0.86 ± 0.04*	0.85 ± 0.02
3	Lipid profile				
	T-CH	108.58 ± 8.96	171.26 ± 8.89	142.31 ± 2.2*	115.58 ± 4.44*
	HDL-CH	40.23 ± 0.83	35.47 ± 0.37	38.97 ± 0.38	39.1 ± 1.63
	TG	147.96 ± 6.9	192.37 ± 5.45	153.9 ± 3.93*	154.44 ± 5.07*
4	Other blood parameters				
	Haemoglobin	14.7 ± 0.73	11.2 ± 0.33	13.37 ± 0.24*	13.7 ± 0.39*
	Erythrocytes	4.47 ± 0.41	2.52 ± 0.21	3.56 ± 0.57	3.34 ± 0.23*
	Leukocytes	8.7 ± 0.50	6.7 ± 0.62	7.4 ± 1.03	8.44 ± 0.26*
	Neutrophils	5.6 ± 0.5	4.2 ± 0.53	4.9 ± 0.73	5.27 ± 0.39
	Eosinophils	0.1 ± 0.02	0.2 ± 0.04	0.1 ± 0.04	0.11 ± 0.02
	Lympho-cytes	2.5 ± 0.13	1.7 ± 0.12	2.15 ± 0.31	2.82 ± 0.16*
	Monocytes	0.3 ± 0.04	0.4 ± 0.04	0.2 ± 0.06	0.42 ± 0.02

n = 5 in each group, values are mean ± s.e.m.
*$P < 0.05$ compared with diabetic control group (ANOVA followed by Dunnett's test).

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Antibacterial and resistance: modifying effects of *Mezoneuron benthamianum*

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The ever increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem resulting in the need for novel antibiotic prototypes. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most problematic bacteria to treat in patients and eradicate from hospitals. In the UK alone, there have been increments in the number of death certificates citing MRSA, with 47 citations in 1993 rising to 398 in 1998 (Crowcroft & Catchpole 2002). Multidrug resistance (MDR) pumps of MRSA protect the cells from antibiotics such as fluoroquinolones, tetracycline and a number of amphipathic cations. Certain natural products, for example reserpine, have been found to be able to block these efflux pumps (Gibbons 2004) and thereby reduce the MIC of norfloxacin and other antibiotics. Such compounds could restore the efficacy of antibiotics against which resistance has arisen. The root bark of *Mezoneuron benthamianum* Baill. (Caesalpinaceae) is used in Ghanaian traditional medicine for the treatment of wounds and other dermal infections. Bioactivity-guided fractionation of the pet. spirit extract led to the isola-

Table 1 Antibacterial susceptibility of test strains in the absence and presence of 10 μg/ml of R2 and R3 and 20 μg/ml reserpine (a naturally occurring MDR efflux inhibitor serving as a standard resistance modulator), n = 3

Antibacterial agent	MIC of test strain expressing the indicated efflux protein	
	SA 1199B (NorA)	XU 212(TetK)
Norfloxacin	32	NT
+R2	2	
+R3	4	
+Reserpine	32	
Tetracycline	NT	128
+R2		16
+R3		32
+Reserpine		32

tion of R1-R5, cassane-type diterpenes whose activities were tested against various bacteria and three strains of resistant *Staphylococcus aureus* possessing the multidrug efflux pumps NorA, TetK and MsrA (SA1199B, XU212 and RN4220). Addition of R2 and R3 to the growth medium at 10 μg/ml resulted in a 16-fold and 8-fold (Norfloxacin) and 8-fold and 4-fold (Tetracycline) potentiation of activities in both compounds respectively (Table 1). R1, R4 and R5 however were not active against the efflux pumps but showed various degrees of activities against other bacteria including some strains of *Mycobacteria*. All compounds had no effect on the erythromycin-resistant *Staphylococcus aureus*.

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In-vitro production of berberine in cultures of *Berberis aristata* and pharmacological investigation for anti-hepatotoxic activity

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Berberis aristata (family: Berberidaceae), a cold climatic plant of high altitude, is a source of berberine (isoquinoline alkaloid), and is used extensively in South Asian Traditional System of Medicines, particularly its roots, stem bark and fruits as tonic for liver and heart (Agarwal et al 2005). *B. aristata* has not been studied utilizing plant tissue culture techniques for in-vitro production of berberine, a compound of commercial interest all over the world because of its medicinal value, but it contains a low concentration of berberine, so the present investigation was aimed to develop standardised protocols for in-vitro enhanced production of berberine, establishment of mechanism based anti-hepatotoxic activity of various culture extracts and purified berberine. For callus initiation, excised leaf and stem explants of matured plant were cultured in-vitro on Murashige and Skoog's (MS) medium supplemented with different combination and concentration of plant growth regulators under controlled condition of temperature (25 ± 2°C), light (1600 lux) and humidity (65%) with day/night regime (16/8 h). For suspension culture studies, calli of fourth passage were cultured on agar free medium with same hormonal combinations. Berberine was isolated, purified, characterized and quantitatively estimated in cultures by HPLC analysis (Chang & Yi-Li 1991). For surface sterilisation of explants, silver nitrate solution (2%) with a few drops of tween 80 and contact time of 5 min was found most effective. The MS medium with 2,-4 dichloro-phenoxyacetic acid (2,4-D) either alone or in combination with low concentration of 6-benzyl adenine (6-BA) were found favourable for initiation and development of leaf and stem callus. Medium with 2,4-D (2ppm), and 6-BA (0.5 ppm) showed best callus initiation while medium with 2,4-D (3 ppm), IIA (1 ppm) and 6-BA (1 ppm) alongwith ascorbic acid (1 gl^{-1}) showed best development of calli. For suspension culture same hormonal combinations devoid of agar were found suitable. To induce hepatotoxicity in albino Wistar rats, carbon–tetrachloride (CCl_4) was administered orally or intraperitoneally. Purified berberine and extracts were administered orally and intraperitonealy at different doses for one week. CCl_4 (1.5 ml/kg/day p.o. and 0.5 ml/kg/day i.p.) produced severe liver damage marked by increase in serum liver transaminases, albumin, total protein, billirubin and alkaline phosphatase. Assessed biochemical parameters were correlated with hepatic lesions produced which include hepatic necrosis, degeneration, broad infiltration of lymphocytes and kupffer cells. One week treatment with extracts at different doses (100 mg/kg/day p.o.; 250 mg/kg/day p.o.) and berberine (10 mg/kg/day p.o.; 5 mg/kg/day i.p.) significantly alleviated serum enzyme activities and liver body weight ratio. Histopathology showed reversal effects. Effects were compared with commercially procured berberine (Sigma; 10 mg/kg/day p.o.; 5 mg/kg i.p.) and standard drug i.e. Silymarin (100 mg/kg/day p.o.). Purified berberine as well as extracts showed remarkable anti-hepatotoxic activity. Moreover, suspension culture extracts produced better than callus culture extracts due to high berberine content. Berberine possesses a significant anti-hepatotoxic property. Its pharmacodynamics include inhibition of Phopholipase A2, alleviation of lipid (LDL), cholesterol, and triglycerides, attenuation of glutathione, increased DNA repair synthesis and anti-oxidant property.

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Antibacterial and wound healing activity of *Paullinia pinnata* L.

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In traditional medicine in Ghana, *Paullinia pinnata* (Sapindaceae) is used for the treatment of wounds and microbial infections. The objective of this study is to assess the antimicrobial, antioxidant and fibroblast proliferation properties of the plant which may underline its wound healing properties. Bioassay-guided fractionation and isolation of methanol extract led to the isolation of seven compounds K1, K2, K3, K5 K9, K10 and K11 from the active fractions (petroleum ether and chloroform) using column chromatography and thin layer chromatography. These were assessed for their antibacterial activities against different strains of *Staphylococcus aureus* (XU212 (TetK), RN4220 (MsrA) and SA1199B (NorA)) possessing efflux mechanism of resistance (Oluwatuyi et al 2004). The antioxidant and fibroblast stimulation action of the crude extract and the isolated compounds were assessed by their ability to scavenge DPPH radical (Yoshida et al 1989) and stimulate the growth of human dermal fibroblast cells (142BR) (Houghton et al 2005), respectively. All compounds (except K5 and K10) had antibacterial effects with minimum inhibitory concentrations (MIC) in the range 1–256 μg mL^{-1}, the most active being K11 with MIC in the range 1–4 μg mL^{-1}. Incorporation of K11 in the bacterial growth medium at 0.1 μg mL^{-1} caused an 8-fold (norfloxacin), 256-fold (tetracycline) and 712-fold (erythromycin) potentiation of activities against SA1199B, XU212 and RN4220, respectively. The crude methanol extract had a strong radical scavenging activity (SC_{50} 3.8 μg mL^{-1} compared with 21.4 μg mL^{-1} of L-ascorbic acid, a known antioxidant widely believed to promote wound healing) while the isolated compounds had weak activity (SC_{50} in the range 26.2–500 μg mL^{-1}) (Table 1). The crude methanol extract also showed a strong fibroblast stimulatory action (48% over the positive control) at 50 μg mL^{-1}. Two compounds K5 and K10 showed fibroblast stimulatory activity with K10 being strongest (34% over the positive control). These findings strongly support the folkloric use of the plant as a natural health product in wound healing. Identification of the compounds is currently underway in our laboratories.

Table 1 DPPH scavenging activity of methanol extracts and compounds

Compound/Extract	SC50 (μg mL^{-1})
K1	500.3 ± 1.3
K5	500.8 ± 0.7
K10	284.7 ± 0.9
K9	389.3 ± 2.1
K11	26.1 ± 0.4
Methanol extract	3.8 ± 0.3
L-Ascorbic acid	21.1 ± 0.6

The scavenging effects were expressed as the percentage inhibition (mean ± s.d., n = 12) compared with the blank.

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Evaluation of some common medicinal plants used by herbalists in Eastern Province, Kenya

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Traditional medicine has been used throughout the world to treat various ailments. Over 80% of the population in the Third World, countries use herbal medicine (Miller 1990). In 1990, the World Health Organization estimated that one traditional doctor treated 500 people as opposed to one conventional doctor for every 40 000 patients. Hence, there is need to promote herbalists and traditional medical practices. The latter should go hand in hand with regulation and evaluation of herbal treatment to avoid the administration of dangerous concoctions. Evaluation tests are done to authenticate the medicinal plants in use by recognized bodies such as universities or research institutions. Scientific proof and clinical validation of herbal formulations is achieved by various methods: chemical standardization, biological assays, animal models and clinical trials (Yuan et al 2000). Bacterial infections are common in the tropics and medicinal plants are widely used in African communities

to treat bacterial infections (Sofowora 1993). In this study medicinal plants commonly used by selected herbalists in Mbeere and Embu districts of Eastern province were evaluated for their inhibition strength to three selected strains of bacteria. Twenty-seven plants commonly used by the herbalists were authenticated by a taxonomist. The plant parts used were dried in the shade, chopped, and ground to a fine powder. A hot water infusion (1.0 g powder extracted with 100 ml) was prepared to be used for the tests. Isolates of three bacteria species were obtained from a medical research centre and the required suspension of bacteria was prepared (equivalent to McFarland standard 1 (1×10^8 CFUs/ml) in 0.85% NaCl $_{(aq)}$ and adjusted by the standard plate count method). Six-millimeter sterile discs were dipped into the aqueous sample extracts and placed on cultured pathogenic bacteria on agar plates and incubated at 37°C. The diameter of inhibition zone of bacterial growth was measured after 24 h. The sensitivity of *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* to the 27 infusions were determined in triplicate. This was repeated using commercial discs of tetracycline (100 μg), streptomycin (25 μg) sulphamethoxazole (200 μg) cotrimoxazole (25 μg) and gentamicin (10 μg) as positive controls. All 27 plant extracts (infusion) investigated showed sensitivity against *E. coli* with inhibition zone diameters ranging from 5.8–10.8 mm. *Terminalia brownii* gave the highest zones of inhibition against *E.coli* and *S. aureus. Vernonia lasiopus* and *Tithonia diversifolia* did not show sensitivity to *S. aureus* and *B. subtilis*, respectively. Eighteen and sixteen plant extracts showed sensitivity of greater than 10 mm against *S. aureus* and *B. subtilis*, respectively. All control discs gave zones of inhibition of 10–24 mm, which were higher than for the extracts. In conclusion, all the medicinal plants were effective against bacterial strains tested except two plants, which were not sensitive to *S. aureus* and *B. subtilis*.

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Glycyrrhizin production in crown gall of *Abrus precatorius* L. (Indian liquorice)

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Agrobacterium tumefaciens is nature's own genetic engineer. It has been attempted for engineering of secondary metabolism in pharmaceutically important plants in the last few years. A virulent strain of *Agrobacterium tumefaciens* causes a disease in susceptible dicotyledonous plants characterized by uncontrolled growth of plant cell known as crown gall found to be a source of therapeutically active compounds or novel secondary metabolite (Stahlhut 1994). Glycyrrhizin (Glycyrrhizic acid), a triterpenoid saponin is an important pharmaceutical being used as natural sweetener, anti-inflammatory, hepatoprotective, immmunomodulatory and radio protective agent. Glycyrrhizin is a chief constituent of *Glycyrrhiza glabra* and also present in *Glycyrrhiza uralensis, Abrus precatorious* L., *Podophyllum vulgare*. Cell suspension cultures of *G. glabra* produce no detectable amount of glycyrrhizin (Hayashi et al 1988). This investigation is the first report on *Agrobacterium tumefaciens* mediated genetic transformation of *Abrus precatorious* L. and plant was found to be susceptible for in vitro and in vivo tumour induction. Leaf discs and stems from green house and in vitro grown *Abrus precatorious* L were used for transformation studies. Transgenic cell lines were established following the infection with wild Ti strains of *A. tumefaciens* MTCC 2250, 2251 and disarmed *Agrobacterium tumefaciens* strain LBA 4404/ p CAMBIA 1305.1. Genetic transformation was confirmed by opine assay and histochemical staining of transformed tissue. Crown gall cells shows vigorous growth on hormone free MS or GB-5 media and exponential phase displayed between 6–10th days of growth cycle. Crown gall cell shows 60–70 fold increases in biomass over untransformed cells. After 45 days of infection crown gall cell lines were maintained on MS media supplemented with NAA 1 mg/l and Kn 3 mg/l (S1) and MS media without plant growth regulators (S2) under 24 h photoperiod with cool white fluorescent light of 25 μmol/m^2/s^1 at 25 ± 1°C. Glycyrrhizin was determined in in vitro and in vivo samples by HPTLC (Chouhan et al 1998). Highest glycyrrhizin production was 0.2–0.4% ($P < 0.05$) in suspension culture of crown gall cells of *Agrobacterium tumefaciens* MTCC 2251 and 2250 at 28th days of growth cycle. Presence of glycyrrhizin in crown gall cells indicates possibility of commercially viable in vitro production system, which can be, explored for scaling up of glycyrrhizin production through bioreactor.

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Asarones in *Acorus calamus* and their acetylcholinesterase inhibition

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Acorus calamus L. (Araceae) is a semi-aquatic, perennial, aromatic herb with creeping rhizomes. The rhizome has many ethnomedicinal uses, including that of helping ailing memory in older people (Howes & Houghton 2003). The major chemical constituents of *A. calamus* oil are β- and α-asarone. The dried and pulverized rhizomes of the plant (600 g) were extracted with n-hexane. After removal of the hexane, the essential oil obtained was dried over anhydrous sodium sulphate to give 5.7 g of a greenish-brown essential oil (0.95% w/w). The asarones were obtained from the oil by prep TLC (silica gel/toluene:ethyl acetate 93:7, visualising with UV light 254 nm; R_f 0.46). The compounds were identified by comparison of their spectroscopic data with literature values. The quantitative estimation of the two asarones was performed using GC (Varian 3400 programmable capillary GC, D.B.5 Wax capillary column (30m × 0.32 mm i.d., film thickness 0.25 μm). Oven temperature was programmed at 140–180°C, at 3°C/min and held isothermally at 180°C for 7.67 min. Injector temperature was 210°C; Detector temperature was 250°C [FID]; carrier gas Helium. 2 μL samples were injected with the Frit-splitter at ratio 12:1. The oil was found to contain 52.33% w/ β-asarone and 1.03% w/w α-asarone. The oil of Indian *Acorus calamus* rhizomes and the two isolated compounds were tested for in vitro AChE inhibitory activity based on Ellman's method in 96-well micro plates using acetylcholinesterase (AChE) from bovine erythrocytes (Perry et al 2000), using physostigmine as a standard. Triplicate determinations were carried out. The oil gave a strong AChE inhibition (IC_{50} 106.75 ± 8.08 μg/ml), as did β-asarone (IC_{50} 3.33 ± 0.02 μM) and α -asarone (IC_{50} 46.38 ± 2.69 μM), respectively. Physostigmine gave IC_{50} value of 0.28 ± 0.015 μM. β-asarone is the constituent with the stronger activity and was present in greater amounts in the oil so appears to contribute most to the AChE inhibition of *A. calamus* oil. This activity may explain the traditional use of *A. calamus* rhizome for failing memory.

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Acetylcholinesterase inhibitory activity of Indian plants used to treat failing memory

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The major therapeutic approach to symptomatic treatment of Alzheimers disease in recent years has been the use of acetylcholinesterase (AChE) inhibitors such as rivastigmine and galantamine. Cognitive decline is associated with low levels of ACh and the use of AChE inhibitors raises these levels. A variety of plants from different cultural traditions used to help mental function in old age has been tested and found to have AChE inhibitory activity (Houghton & Howes 2005). The Ayurvedic tradition in Indian medicine utilizes a variety of plants of treating decline in mental function in old age so it was thought pertinent to test some of these plants for AChE inhibitory effects. Five plant species were used (Table 1) and extracted with 70% ethanol. A range of different

Table 1 Indian medicinal plants and IC_{50} values (μg/ml) for AChE

Species	Part used	IC_{50} (ug mL^{-1})
Centella asiatica (L.) Urban Apiaceae	Whole plant	106.6 ± 9.9
Evolvulas alsinoides L. Convolvulaceae	Whole plant	141.8 ± 16.3
Nardostachys jatamansii DC. Valerianaceae	Rhizome	130.1 ± 13.0
Nelumbo nucifera Gaertn. Nymphaeaceae	Rhizome	185.6 ± 21.2
Myristica fragrans Houtt. Myristicaceae	Seeds	133.3 ± 11.3
Physostigmine Positive control		0.076 ± 0.004

Means ± s.e.m., n = 3.

concentrations of the extracts was tested using the Ellman reaction (Perry et al 2000). This test relies on the measurement of the release of thiocholine, using acetylthiocholine as the substrate for the AChE. The percentage inhibition of AChE after three minutes was determined for each extract. When these values were plotted against concentration, the IC_{50} value for each extract could be calculated using Graphpad Prism software. Results are shown in Table 1. Although the activity was weak compared with the positive control, the activities displayed are similar to those shown by other plants with an ethnopharmacological use in cognitive decline (Houghton & Howes 2005). Work is in progress to determine the identity of the compounds responsible for activity.

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Discrimination between 'drug type' cannabis and hemp using Near Infrared Spectroscopy

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The identification and quality control of plant material can be carried out using Near Infrared (NIR) Spectroscopy. It differs from other techniques traditionally used to profile plant material, as it is not separative and the spectrum obtained is representative of the sample as a whole. The technique is also non-destructive and little or no sample preparation is required. After a spectral database has been constructed, multivariate analysis and chemometric techniques can be used to show similarities or differences between the spectra of different samples. The spectrum of the incoming 'test' sample is compared to the database and may be assigned to an existing group of samples if it falls within a pre-determined threshold, or a 'no match' may be given. The main psychoactive component of 'drug type' cannabis, Tetrahydrocannabinol (THC), is present only at very low levels in hemp, which is often used in the food and textile industries, although other cannabinoids may be present. THC is present in higher amounts in cannabis resin and the flowering tops of plants and at lower levels in the leaf. Extracts of 'drug type' cannabis will differ in their THC content, as this depends on the solvent used; the cannabinoids are soluble in non polar solvents such as ethyl acetate and heptane, but less so in ethanol and/or water. Fresh samples have higher levels of THC than older samples, as THC degrades with time to cannabinol (CBN). Different plant materials, including 'drug type' cannabis and hemp in the form of flowering tops or leaf were scanned on a FOSS NIRSystems 6500 spectrophotometer with Rapid Content Sampler module in clear glass vials using Vision software. The scanning wavelength range was 1100–2500 nm, but for all analyses, the water region between 1800 nm and 2000 nm was removed. This was to eliminate the discrimination of samples based on their water content. An average of at least three spectra was used for all samples and Standard Normal Variate 2^{nd} derivative spectra were used for all analyses. Samples were assigned as either 'high THC' or 'low THC' in the spectral library and the use of spectral correlation methods allowed for the correct identification of all samples. Principal Component Analysis (PCA) (The Unscrambler software) was also carried out on the spectral database and the scores plot discriminated between the 'high' and the 'low' THC content samples. The first Principal Component loading correlated with the NIR spectrum of THC, further supporting the evidence that the differences between the two sets of samples were due to the THC content. The library was 'interrogated' with further samples which included older samples of 'drug type' cannabis and material with the cannabinoids removed to varying degrees by solvent extraction. The scores plots obtained were consistent with their THC content. This demonstrated the robustness of the analytical models used to discriminate between the THC-rich and hemp forms of cannabis.