

ORIGINAL ARTICLE

Open Access

# Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy

Sneha Das, Neeru Vasudeva\* and Sunil Sharma

## Abstract

**Background:** *Macrotyloma uniflorum* Linn (Fabaceae) is a herbaceous plant with annual branches. It is used in kidney stones, inflamed joints, fever, musculoskeletal disorders, sinus wounds and localized abdominal tumors. It is reported as an antioxidant and nutraceutical (forage and food). GC-MS analysis of ethanol extract has led to identification of twenty-eight compounds from *M. uniflorum* by comparison of their retention indices and mass spectra fragmentation patterns with those stored on the GC-MS computer library.

**Results:** The main constituents identified were mome inositol, ethyl alpha-d-glucopyranoside, n-hexadecanoic acid, linoleic acid (9, 12-octadecadienoic acid), its esters and ethyl derivatives, Vitamin E, stigmasterol and 3-beta-stigmast-5-en-3-ol.

**Conclusions:** The extracts are rich in linoleic acid and its esters, mome inositol and ethyl alpha-d-glucopyranoside; therefore, this plant can be medicinally beneficial as an antioxidant, in diabetes and its related disorders.

**Keywords:** GC-MS; *Macrotyloma uniflorum*; Chemical constituents; Retention indices

## Background

*Macrotyloma uniflorum* (synonym: *Dolichos biflorus* Linn.) belonging to the family Fabaceae is commonly known as Kulthi in Hindi and horse gram in English. It is a herbaceous plant with annual branches, sub-erect or twining, leaflets of 2.5 to 5 cm. Its seed is 6 to 8 mm long and 3 to 4 mm broad. The seeds are trapezoidal oblong or somewhat rounded in shape and pale to dark reddish brown or orange brown or all black in colour. The genus *Macrotyloma* comprises about 25 species, most of which are restricted to Africa of which four have been identified as *M. uniflorum*, *M. stenocarpum*, *M. verrucosum* and *M. benadirianum* [1]. Traditionally, it has been widely used in the treatment of kidney stones, inflamed joints, fever, musculoskeletal disorders, sinus wounds and localized abdominal tumors [2,3]. Experimentally, the seeds are reported as hepato-protective, diuretic and antioxidant [4-6]. To the best of the authors' knowledge, no published literature exists about the chemical contents of the ethanol extract

of *M. uniflorum*. Thus it was planned to carry out GC-MS analysis of ethanol extract of the seeds of *M. uniflorum*.

## Methods

### Preparation of crude extract

The seeds (1 Kg) were coarsely powdered and defatted with petroleum ether (60°C to 80°C) for 7 days by cold maceration. The fat-exhausted drug was further extracted with ethanol (95% v/v) by soxhlation for 72 h. The extract was concentrated in a rotary vacuum evaporator to yield 25.0% w/w of dark-brown-coloured extract. The ethanol extract of seeds was diluted with ethanol and filtered with Whatman No. 42 to obtain a particle-free extract for analysis by GC-MS.

### GC-MS analysis

The extract was directly used for the analysis. GC-MS was carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and auto injector (AOC-20i), equipped with mass selective detector, having ion source temperature of 230°C, interface temperature of 260°C, a solvent cut time of 2.50 min threshold of 1,000 eV and mass range of 40 to 650 m/z.

\* Correspondence: neeruvvasudeva@gmail.com  
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana 125001, India

Compounds were separated using a Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 μm (film thickness). The split mode was used at a ratio of 10:1. The temperature of the injector was initialized to 250°C, having a split injection mode. The temperature was programmed from 100°C (3 min), then further increased to 280°C at a ramp rate of 10°C/min (19 min hold).

Helium (>99.999%) was used as the carrier gas at a linear flow velocity of 40.9 cm/s. The debit of gas (helium) vector was fixed to 16.3 mL/min, with a total flow of 1.21 mL/min. The volume of injected sample was 1.0 μL of ethanol extract. The components were identified by comparison of their retention indices (RI) relative to homologous alkane series (purchased from Sigma, St. Louis, USA) and by comparison of their mass

spectral fragmentation patterns with those data provided in WILEY8.LIB, NIST08.LIB, NIST08s.LIB and NIST.LIB. Identification was assumed when a good match of mass spectrum and RI was achieved.

## Results and discussion

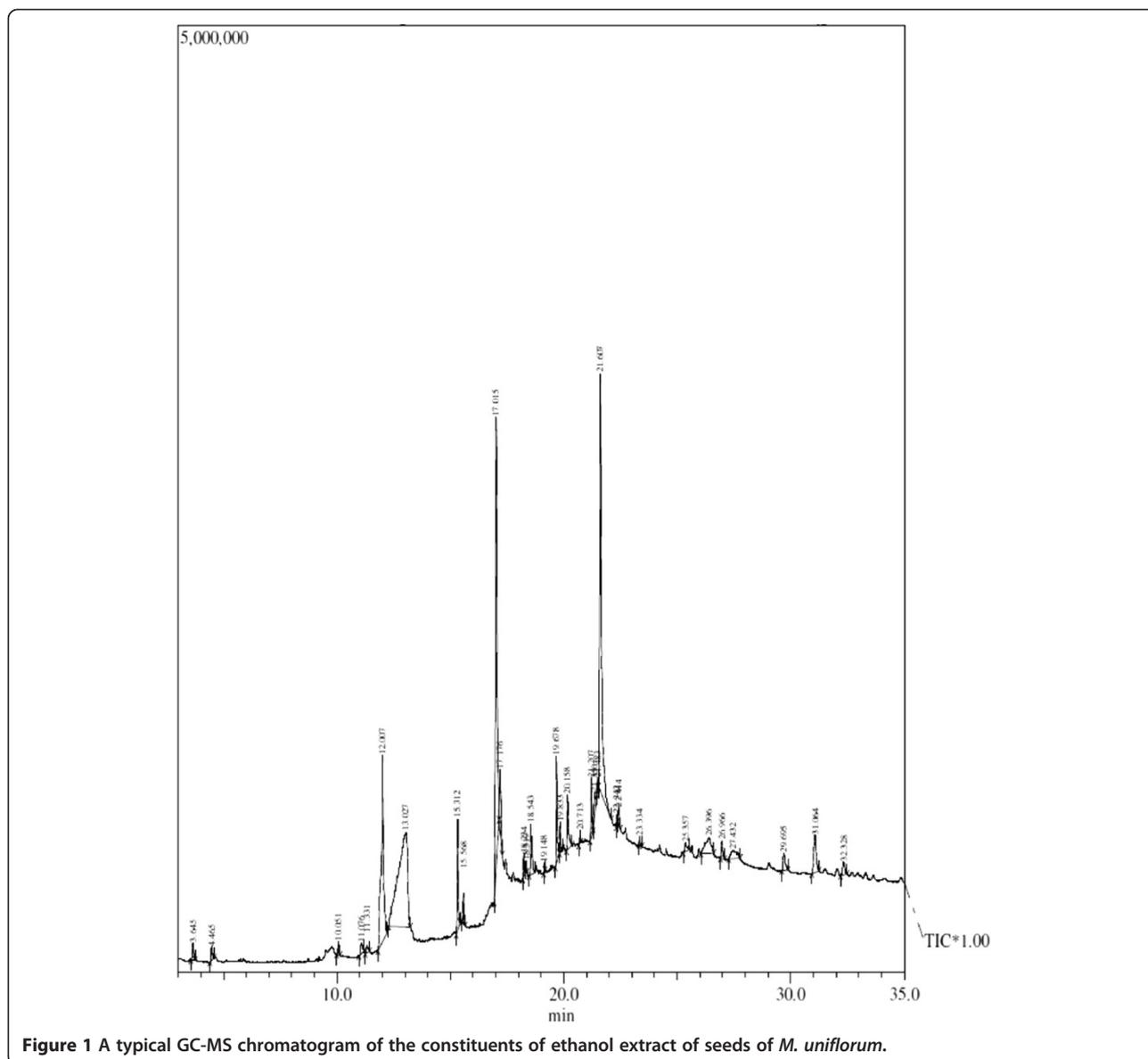
The seeds were purchased from Bhagalpur district, Bihar, India, and identified by Dr. K. C. Bhatt, NBPGR, New Delhi. A voucher specimen (PGS-13-02) has been deposited in the Department of Pharmaceutical Sciences (Pharmacognosy Division), Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India.

## Chemical composition

GC-MS analysis of ethanol extract led to the identification of twenty-eight compounds from *M. uniflorum* (Table 1, Figure 1). The main constituents identified were two

**Table 1 Chemical composition of ethanol extract of *M. uniflorum* seed**

Peak	Retention time	Retention indices	Area%	Name
1	3.645	1,056	0.67	Benzeneacetaldehyde
2	4.465	1,116	0.51	Benzeneethanamine
3	10.051	1,499	0.27	L-Phenylalanine, ethyl ester
4	11.076	1,580	0.53	2-(1-methyl-2-propenyl)bicyclo[2.2.1]heptane
5	11.331	1,602	0.39	1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl)
6	12.007	1,660	11.14	Ethyl .alpha.-d-glucopyranoside
7	13.027	1,750	23.24	Mome inositol
8	15.312	1,971	2.76	n-Hexadecanoic acid
9	15.568	1,996	0.49	Heptadecanoic acid, ethyl ester
10	17.015	2,151	19.79	9,12-Octadecadienoic acid (Z,Z)
11	17.176	2,169	1.61	Ethyl (9Z,12Z)-9,12-octadecadienoate
12	18.319	2,300	0.12	Heptadecane, 3-methyl
13	18.543	2,327	1.88	Octanamide, N-(2-hydroxyethyl)
14	19.678	2,465	3.18	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
15	19.833	2,484	0.60	(R)14-Methyl-8-hexadecyn-1-ol
16	20.158	2,526	1.77	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
17	21.207	2,662	1.27	1-Cyclohexyldimethylsilyloxybutane
18	21.483	2,698	0.22	Eicosane
19	21.607	2,712	19.49	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
20	22.342	2,798	0.50	Heneicosane
21	22.414	2,805	0.33	9-Methyl-10,12-hexadecadien-1-ol acetate
22	23.334	2,897	0.19	Hexatriacontane
23	26.396	3,116	2.42	i-Propyl 9,12-octadecadienoate
24	26.966	3,154	0.72	Vitamin E
25	27.432	3,181	1.76	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy
26	29.695	3,290	0.97	Stigmasterol
27	31.064	3,355	2.42	Stigmast-5-en-3-ol, (3 beta)
28	32.328	3,413	0.74	(-)-Isolongifolol, acetate



polysaccharides namely mome inositol and ethyl alpha-d-glucopyranoside. A number of fatty acids and their esters have also been identified; they were n-hexadecanoic acid, 9, 12-octadecadienoic acid and its esters. Phytosterols, namely stigmasterol and 3- $\beta$ -stigmast-5-en-3-ol, were present in traces. Other compounds present were 3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester, heneicosane and vitamin E. literature survey showed that Dolichin A and B and pyroglutaminyglutamine along with some flavonoids were isolated from this plant. Seeds of *M. uniflorum* contain lectins, glycoprotein, agglutinin, an anti-A phytoagglutinin, four glycosidase enzymes, allantoinase and a strong diuretic dipeptide, pyroglutamylglutamine. The seeds are rich source of the enzyme urease and also contain  $\beta$ -sitosterol [7].

## Conclusions

Mome inositol, one of the major components of extract of *M. uniflorum*, is reported as anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic and a sweetener. n-hexadecanoic acids act as a 5-alpha-reductase inhibitor, a hemolytic agent and an antioxidant [8]. (3 $\beta$ )-stigmast-5-en-3-ol has shown an insulin-like effect, that is, stimulating glucose transport apart from its existing cholesterol-lowering efficacy. Therefore, it can play a beneficial role as an antidiabetic agent [9]. Animal studies have revealed that linoleic acid is converted to gamma linoleic acid in the body and can prevent chemically induced diabetes while restoring normal antioxidant status in tissues [10]. It can also prevent diabetic neuropathy, a painful condition resulting from exposure of nerves to

high glucose levels [11]. Hence, the plant can be utilized as a natural sweetener, anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic, antioxidant and antidiabetic.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

SD has carried out all practical work and wrote the manuscript. NV and SS supervised all the work including the designing and drafting of the manuscript. All authors read and approved the final manuscript.

#### Authors' information

Sneha Das is PhD research scholar pursuing her doctorate under supervision of Professor Neeru Vasudeva and Professor Sunil Sharma at Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India, 125001.

#### Acknowledgements

We are grateful to Department of Science and Technology, New Delhi, India, under the Ministry of Science and Technology, New Delhi, India, and the Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, for providing fellowship and infrastructure, respectively to Sneha Das for carrying out the research. Also we would like to thank Dr. Ajay Kumar, scientist at AIRF, JNU, Delhi, for helping us perform GC-MS.

Received: 28 September 2014 Accepted: 20 October 2014

Published online: 02 December 2014

#### References

1. Mehra A, Upadhyaya M (2013) *Macrotyloma uniflorum* Lam traditional crop of kumaun himalaya and ethnobotanical perspectives. *Int J Agric Food Sci* 3(4):148–150
2. Muthu AK, Sethupathy S, Manavalan R, Karar PK (2005) Hypolipidemic effect of methanolic extract of *Dolichos biflorus* Linn in high fat diet fed rats. *Ind J Exp Biol* 43:522
3. Muthu AK, Sethupathy S, Manavalan R, Karar PK (2006) Antioxidant potential of methanolic extract of *Dolichos biflorus* Linn. in high fat diet fed rabbits. *Ind J Pharmacol* 38:131
4. Parmar HB, Das SK, Gohil KJ (2012) Hepatoprotective activity of *Macrotyloma uniflorum* seed extract on paracetamol and d-galactosamine induced liver toxicity in Albino rats. *IJPR* 2(2):86–91
5. Ravishankar K, Priya PSV (2012) Evaluation of diuretic effect of ethanolic seed extracts of *Macrotyloma uniflorum* and *Cucumis melo* in rats. *Int J Pharm Bio Sci* 3(3):251–255
6. Ravishankar K, Priya PSV (2012) In vitro antioxidant activity of ethanolic seed extracts of *Macrotyloma uniflorum* and *Cucumis melo* for therapeutic potential. *IJRPC* 2(2):442–445
7. Ghani A (2003) Medicinal Plants of Bangladesh with Chemical Constituents and Uses. Asiatic Society of Bangladesh, Dhaka, Bangladesh, p 181
8. Kumar NR, Reddy JS, Gopikrishna G, Solomon KA (2012) GC-MS determination of bioactive constituents of *Cycas beddomei* cones. *Int J Pharm Bio Sci* 3(3):344–350
9. Sujatha S, Anand S, Sangeetha KN, Shilpa K, Lakshmi J, Balakrishnan A, Lakshmi BS (2010) Biological evaluation of (3β)-STIGMAST-5-EN-3-OL as potent anti-diabetic agent in regulating glucose transport using in vitro model. *Int J Diabetes Mellitus* 2:101–109
10. Suresh Y, Das UN (2003) Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus: effect of omega-6 fatty acids. *Nutrition* 19(2):93–114
11. Pitel S, Raccah D, Gerbi A, Pieroni G, Vague P, Coste TC (2007) At low doses, a gamma-linolenic acid-lipoic acid conjugate is more effective than docosahexaenoic acid-enriched phospholipids in preventing neuropathy in diabetic rats. *J Nutr* 137(2):368–372

doi:10.1186/s13588-014-0013-y

**Cite this article as:** Das et al.: Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy. *Organic and Medicinal Chemistry Letters* 2014 **4**:13.

**Submit your manuscript to a SpringerOpen® journal and benefit from:**

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](http://springeropen.com)