

GC-MS Analysis of Methanol Extracts of Five Toxic Plant Seed For Detection of Bioactive Compounds

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Abstract

The present work was carried out to explore the qualitative analysis of bioactive phyto-constituents present in methanolic extracts of five toxic plant seeds by using gas chromatography-mass spectrometry. The analysis of extracts revealed the presence of the different biologically active compounds including alkaloids, glycosides, flavonoids, phenols, fatty acid, ester, epoxide, steroids, and protein etc. Forensic toxicological chemistry deals with the chemistry and analysis of various types of poisons including plants. Diagnosing plant poisoning especially their seeds can be difficult and must be quickly done. In conclusion, the recovery of various toxic and therapeutically constituents could be helpful for forensic toxicologist.

Keywords: GC-MS, plant extracts, NIST, formulation

Introduction

Being habitat of wide diversity of traditional herb and herbal products, India is rightly called as the botanical garden of the world ¹. There are approximately 4,00,000 species of vascular plants be present on the Earth. They produce thousands of metabolites but actual composition, function and supplementary other utility of many such phytochemicals are yet to be investigated ². Phytochemicals are plant produced chemical compounds that are generally helpful against various types of pathogens. Some of these can act as poison be used as poisons whereas others for medicinal purpose. Plants synthesize a wide variety of medically (antibacterial and antifungal etc) important chemical compounds, which are classified as primary metabolites and secondary metabolites³⁻⁴. They also show a number of protective functions for human consumers. These bioactive constituents may also act as cardiotoxic and neurotoxic on human beings and livestock ⁵. In the last few decades, the GC-MS has been considered one of the most efficient, easiest and fastest technique to identify the bioactive constituents in both plant and non-plant

species ⁶. The analyses of bioactive constituents in plant material can play an important role in the development, modernization and quality control of herbal formulations ⁷. Considering a valuable addition to plant sciences and pharmacology, the current study was attempted to generate GC-MS based database of photochemicals in methanolic extract of seed of samples of *Thevetia neriifolia*, *Jatropha curcus*, *Abrus precatorius*, *Datura innoxia*, and *Ricinus communis* from Rohtak district of Haryana, India. This study is supposed to be a valuable addition to the literature based on the GC-MS analysis of bioactive compounds in toxic plant seed.

Materials and Methods

Collection and identification of plants: Five toxic plant seed samples of i.e. *Thevetia neriifolia*, *Jatropha curcus*, *Abrus precatorius*, *Datura innoxia*, and *Ricinus communis*, were collected during July- October 2018 from Rohtak district of Haryana. These plants were identified as based on their morphological features as well as molecular signature by using ITS marker in cases of *Thevetia neriifolia* (Accession No MH777018), *Jatropha curcus* (MH923185), *Abrus precatorius* (MH756599), *Datura innoxia* (MH762134) and by using *rbcL* marker *Ricinus communis* (Accession No-MK672874). All voucher specimens were deposited in the forensic chemical laboratory of the Department of Genetics, M.D. University Rohtak, Haryana (India)..

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Preparing of Extracts: All the seed samples of these toxic plants was washed individually with running water and left to dry at room temperature for about a week. Seeds were crushed to powder form and about 60 gram was extracted in 200 ml methanol in Soxhlet apparatus at a temperature of 60°C. Extracts were evaporated to dryness in rotary evaporator and stored at 4°C for further analysis.

GC-MS of Extracts

Phytochemical analysis of five toxic plant seed extracts was carried out using a GC-MS analyzer (BRUKER SCION 436-GC SQ). Extracts were dissolved in methanol (GC grade) and filtered through Whatman TM filter device, pore size 0.2 µm. Helium (99.99%) was used as a carrier gas, at a flow rate of 1 ml per minute in split mode. Restek Rtx®-5 capillary columns (Crossbond® 5% diphenyl/ 95% dimethyl polysiloxane) with 30 m length, 0.25 µm df and 0.25 mm ID column

was used for separation of phytochemicals. Exactly 2 µL of the sample was injected in a column. The injector temperature was 280°C. Oven temperature started at 70°C and held for 2 min and then was raised at a rate of 70°C per minute up to 320°C; hold for 1 min. The temperature of ion sources was maintained at 250°C. The mass spectrum obtained by electron ionization at 70eV and detector operates in scan mode 30 to 500 Da atomic units. Total running time was 30 min including 3 min solvent delay.

Results and Discussion

Results of many phytochemical compound in the seeds of *Thevetia neriifolia*, *Datura innoxia*, *Abrus precatorius*, *Jatropha curcus*, and *Ricinus communis* (Figure 1-5). The interpretation of mass spectra identified bioactive compounds by using the database of the National Institute of Standards and Technology (NIST) installed in GC-MS library.

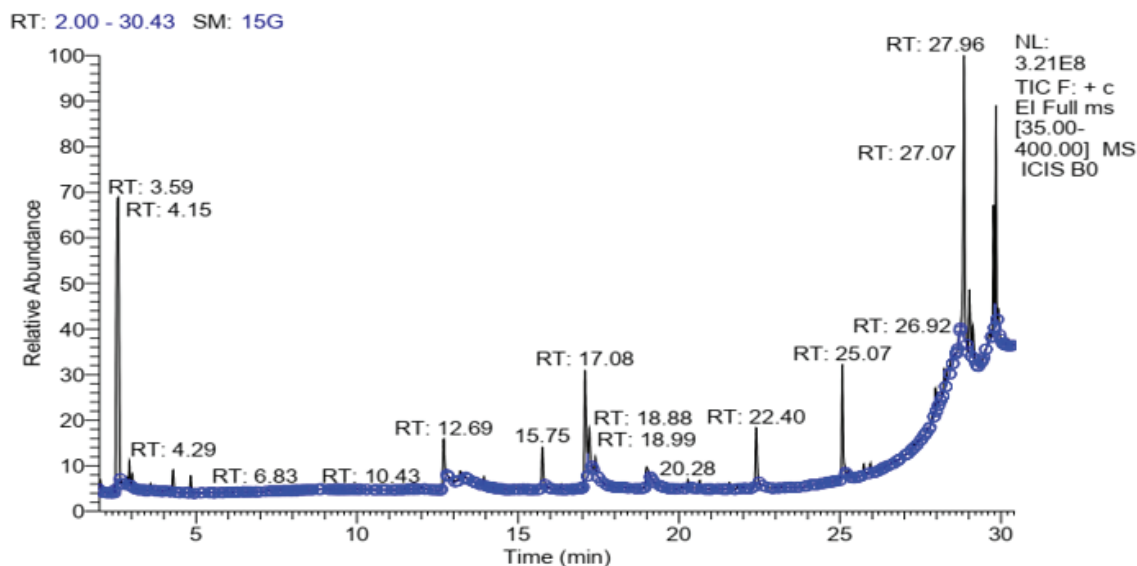


Figure 1: *Thevetia neriifolia*

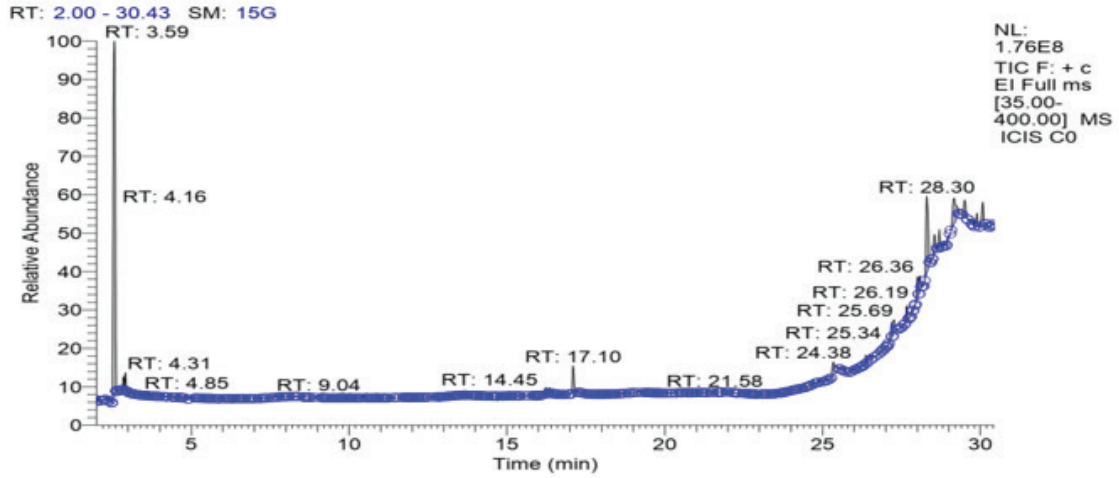


Figure 2: *Datura innoxia*

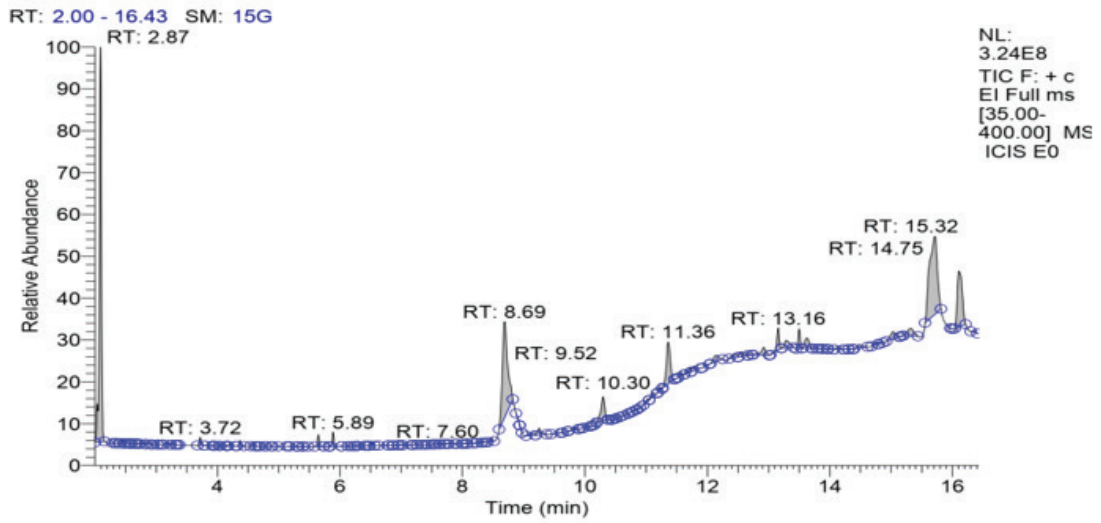


Figure 3: *Abrus precatorius*

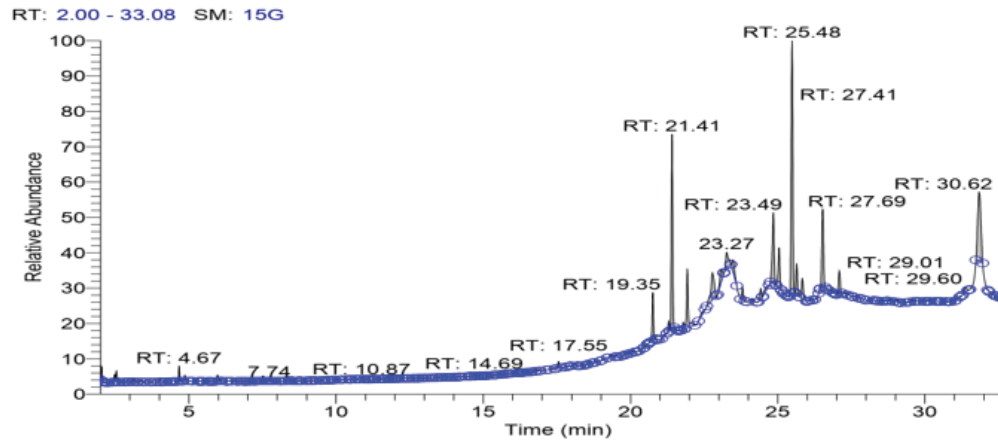


Figure 4: *Jatropha curcus*

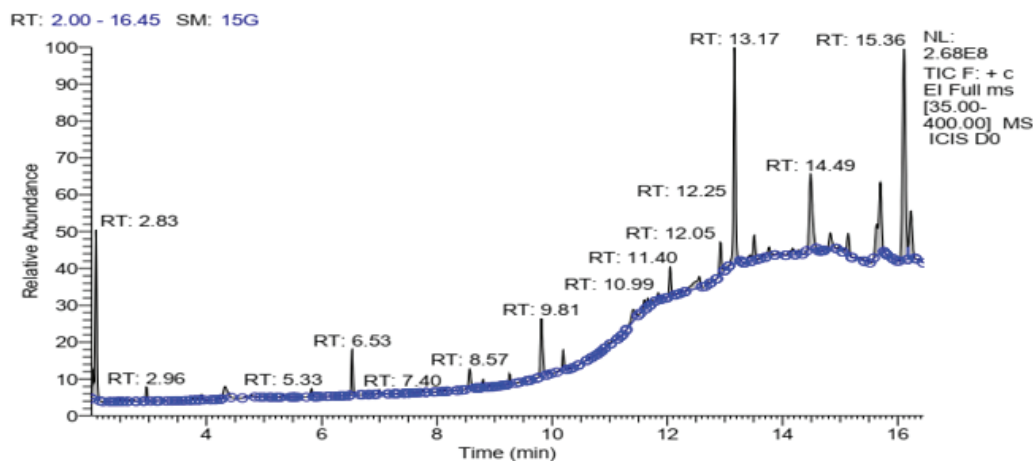


Figure 5: *Ricinus communis*

Figure 1-5: GC-MS chromatogram of methanolic extracts of seeds of selected plants

GC-MS revealed several bioactive compounds in plant seeds materials some of which were used for therapeutic purposes. These bioactive compounds included 9,12-Octadecadienoic acid, ethyl ester (linoleic acid) having a properties of hypocholesterolemic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic, and anti-acne), antimicrobial properties has been reported of compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy, benzaldehyde, 4-Hydroxy-3-Methoxy, dodecanoic acid, octadecanoic acid. Some compounds like tetradecanoic acid hexadecanoic acid, methyl ester and n-Hexadecanoic acid having properties of antioxidant, cancer-preventing, nematicide, larvicidal and repellent activity, pesticide antiandrogenic, hemolytic and antifungal⁸⁻¹¹. Some of the compounds detected by GC-MS of plant extracts were, 3-Butanedione *synonym* diacetyl /di methylglyoxal potential toxic organic compound. It has been associated with several diseases includes Crohn's disease, bronchiolitis obliterans, ulcerative colitis, nonalcoholic fatty liver disease, inborn metabolic disorders (celiac disease). All of this toxicity, this compound has been commonly used as a flavoring agent in artificial butter, and dairy fermented products at the current level in beverages (5 ppm) and food (50 ppm).¹². Literature reported various health issues with 2, 3-butanedione monoxime compound when it enters especially a concern when entering the central nervous system then increase blood pressure with increased heart rate as a result of cardiac arrest¹³. Another important compound has detected 2(3H)-Furanone, 5-methyl which is a lactone

(Butenolides)category commonly used in antimicrobial, antiproliferative, anti-inflammatory activities¹⁴. Besides these therapeutic activities, a toxicological profile had been found in the case of Sesquiterpene lactones which induce toxic syndrome when it directs contact of the dermis in human and non-human¹⁵. Dodecene compound shows toxicity at a high concentration that irritates eyes, nose, throats, and CNS depression when ingested or inhale had reported¹⁶. A toxicity relevancy data of 2-Pentanone, 4-hydroxy-4-methyl with direct contact may cause sensation of pain, slight dizziness, burning, and itching irritation, by inhalation neurological effects, and strong odor sensation reported¹⁷⁻²¹. Literature had mentioned another saturated fatty acid such as myristic acid/1-tetradecanoic *acid* components of phospholipids of the plasma membrane, so even a small quantity of fatty acid is lost as a cell sloughing²². The acute toxicity of myristic acid has been found in rats with the lethal dose > 5g/kg b.w.¹¹⁻¹². Literature observed when it administered in a higher dose, the sign of toxicity had been observed such as depression, excessive salivation and serosanguineous discharge from the muzzle and eyes²². In 1998, JEFCA released a current safety intake value of 1.2 µg/kg b.w./ day in the USA and 2.6 µg/kg b.w./day in Europe.

Misidentification of the nature of compounds that produce some toxicity reported in earlier investigation, most of the secondary metabolites synthesize in plants yielded toxicity in various ways including pyrrolizidine alkaloids, some unusual amino acids (canavanine

and azetidine-2 carboxylic acid) and some analogs of these amino acid such as arginine and proline which is non-protein amino-acids that included into proteins but are present as free forms or act as protective defensive substance⁵. Phorbol esters are a tetracyclic diterpenoid that generally role to promote the action of diacylglycerol, tumor activities and also play a role of protein kinase activator which regulates different signal transduction pathways and other cellular metabolic activities discussed in²³. Octadecanoic acid synonym stearic acid is a long-chain fatty acid with 18 carbon backbones. At high concentration of stearic acid gave a direct correlation to plasma cholesterol and fibrinogen that more promote thrombogenesis than oleic acid. Hence mortality caused due to cardiomyocyte which is a concentration dependence manner²⁴⁻²⁷. Besides the toxicity of the steric acid, it is used as a flavoring agent safety issues at the current level intake of 31 µg/kg/day in U.S.A. and 0.97 µg/kg per day in Europe²⁸. According to HSDB online lauric or dodecanoic acid, a short-chain saturated fatty acid at dose 4.64 and 10.0 g/kg in mice observed depression, mucoid diarrhea, excessive salivation, oily, unkempt fur and serosanguineous discharge from the muzzle and eyes¹⁷. Beside toxicity, lauric acid is use in detergent and flavor purpose but it is quite safe to use 21 µg/kg b.w. per day in the USA and 9.9 µg/kg body weights per day in Europe²⁸. Oleic acid is a C18 cis-monounsaturated fatty acid. It is highly absorbed and stored into adipose tissues or undergoes β-oxidation. For oleic acid catabolism, an additional isomerization pathway is required. It is mainly transported via the lymphatic system and it is reported that oleic acid can also penetrate the skin of rats. Since oleic acid is highly absorbed, it is poorly excreted². Ld 50 in a rat of 74 g/kg²¹. Administration of oleic acid to rats up to 21.5 mL/kg caused neither deaths nor gross lesions at necropsy. However, signs of toxicity, including depression, excessive salivation, diarrhea and serosanguineous discharge from the muzzle and eyes, could be observed²⁹. Rats that received a cream formulation containing 5 % oleic acid at the dose of 5 mL/kg, showed weakness and colored urines and feces³⁰. Another important fatty acid, 2-octanone produced from castor oil through a cracking process. During the process of cracking the ricinoleic acid is cut in two molecules: sebacic acid and 2-octanol Soluble in a most organic solvent or as a raw material as flavoring and fragrance purposes, paint coating, inks adhesives, lubricant, and fuels. Volatile compound combustible, by inhaling cause cough and sour throat, dry skin,

red eyes, by ingestion feeling in burning sensation³¹. Literature mentioned castor oil contains 90% ricinoleic acid and their acute toxicity 0.3-3 mg/animal in guinea pigs which showed pro-inflammatory effects³². A case of allergy due to the presence of propylene glycol ricinoleate in lipstick has reported in the literature. In this case, the main constituents of allergy were confirmed by the positive reaction with a patch test to ester gum and ricinoleic acid presence in the lipstick sample. On the other hand, ricinoleic acid and 12-hydroxystearic acid are being to be considered the main constituents of allergens in hydrogenated castor oil and castor oil, some impurities might be risky and reason for definite allergens³³. Methyl Hexyl Ketone form of colorless transparent liquid derived from castor oil by oxidation of nitric acid 2octanol to 2-octanone followed by further oxidation to a carboxylic acid³⁴. This compound showed low toxicity when it directs contact with skin may cause defatting and irritation of the skin, by inhalation, may produce mild symptoms of eye, nose, and throat. Besides this toxicity, literature reported various application of esters in cosmetics, lubricants, food flavoring, fragrance and detergents, etc³⁵⁻³⁶.

Conclusion

Present work generated a data of bioactive constituents detected by GC-MS in of five toxic plant seeds. The study reports presence of some important components could be useful for the researcher working in the fields of pharmaceutical industries, environment science, and toxicology sciences including forensic toxicology.

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Conflicts of Interest: No conflict of interest.

Ethical Clearance: No ethical issues involved as the study was analytical and did not violent any ethical principles

References

- Nayar MP, Sastry AR. Red data book of Indian plants. 1999.
- Wang S, Alseekh S, Fernie AR, Luo J. The structure and function of major plant metabolite modifications. *Mol Plant*. 2019; 12(7): 899-919.
- Gomathy S, Rathinan K. Identification of insecticidal compounds in *Terminalia arjuna* bark extract using gas chromatography and mass spectroscopic technique. *J Otolaryngology-ENT Res*. 2017; 2: 108-12.
- Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*. 2015; 2(3):251-86.
- Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: Overview. *Curr Trends Biotechnol Pharm*. 2015; 9(3):293-304.
- Kanthal LK, Dey A, Satyavathi K, Bhojaraju P. GCMS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. *Pharmacogn Res*. 2014; 6(1):58-61..
- Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. *J Tradit Complement Med*. 2017; 7(2): 234-44.
- Gavamukulya Y, Abou-Elella F, Wamunyokoli F, El-Shemy HA. GC-MS analysis of bioactive phytochemicals present in ethanolic extracts of leaves of *Annona muricata*: A further evidence for its medicinal diversity. *Pharmacognosy J*. 2015; 7(5).
- Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). *Medicines*. 2016; 3(1):2-9.
- Vijayabaskar G, Elango V. Determination of phytochemicals in *Withania somnifera* and *Smilax china* using GC-MS. *J Pharmacogn Phytochem*. 2018; 7(6): 554-7.
- Burdock GA and Carabin IG. Safety assessment of myristic acid as a food ingredient. *Food Chem Toxicol*. 2007; 45: 517-29.
- Burdock GA. Fenaroli's Handbook of Flavor ingredients. CRC press. 2016.
- Sellin LC, McArdle JJ. Multiple effects of 2, 3°C butanedione monoxime. *Pharmacol Toxicol*. 1994; 74: 305-13. 1994; 74(4-5): 305-13.
- Fattori V, Zarpelon AC, Staurengo-Ferrari L, Borghi SM, Zaninelli TH, Da Costa FB, Alves-Filho JC, Cunha TM, Cunha FQ, Casagrande R, Arakawa NS. Budlein a, a sesquiterpene lactone from *viguiera robusta*, alleviates pain and inflammation in a model of acute gout arthritis in mice. *Front Pharmacol*. 2018; 9:1076.
- Bosco A, Golsteyn RM. Emerging anti-mitotic activities and other bioactivities of sesquiterpene compounds upon human cells. *Mol*. 2017; 22(3):459.
- Bingham E, Cochrissen B, Powell CH. Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. 2001
- HSDB (Hazardous Substances Data Bank), online. Available from: <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?/temp/~OxXdOx:Full>. Consultation date: 2011-12-28.
- Dick RB, Krieg EF, Setzer J and Taylor B. Neurobehavioral effects from acute exposures to methyl isobutyl ketone and methyl ethyl ketone. *Fund Appl Toxicol*. 1992; 19: 453- 473.
- Hazleton Laboratories, Inc., 1965. Human sensory irritation thresholds in five ketones: final report. Sponsored by Esso Research and Engineering Company. Submitted to EPA under TSCA section 8D. EPA Document Number 87-8210936; Fiche No. OTS0206265. As cited by U.S.EPA, 2003
- Hjelm EW, Boman A, Fernström P, Hagberg M and Johanson G. Percutaneous uptake and kinetics of methyl isobutyl ketone (MIBK) in the guinea pig. *Toxicol Letters*. 1991;56: 79 -86.
- Iregren A, Tesarz M, Wigaeus-Hjelm E. Human experimental MIBK exposure: effects on heart rats, performance, and symptoms. *Environ Res*. 1993; 63: 101-8.
- Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. 2005: 422-541.
- Goel G, Makkar HP, Francis G, Becker K. 2007. Phorbol esters: structure, biological activity, and toxicity in animals. *Int J Toxicol*. 2007; 26(4): 279-

- 88.
24. Tholstrup T. Influence of stearic acid on hemostatic risk factors in humans. *Lipids*. 2005; 40(12): 1229-35.
 25. Thijssen MA, Hornstra G, Mensink RP. Stearic, oleic, and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. *J Nutr*. 2005; 135(12): 2805-11.
 26. Li D. 2001. Relationship between the concentrations of plasma phospholipid stearic acid and plasma lipoprotein lipids in healthy men. *Clin Sci*. 2001; 100; 25-32.
 27. Rabkin SW, Lodhia P. Stearic acid-induced cardiac lipotoxicity is independent of cellular lipid and is mitigated by the fatty acids oleic and capric acid but not by the PPAR agonist troglitazone. *Exp Physiol*. 2009; 94: 877-87.
 28. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Evaluation of certain food additives and contaminants. Forty-ninth Meeting of the Joint FAO/WHO. Monograph for Saturated Aliphatic Acyclic Linear Primary Alcohols, Aldehydes and Acids. WHO Food Additives Series.
 29. US Department of Health and Human Services. Hazardous substances data bank (HSDB, online database). National Toxicology Information Program, National Library of Medicine, Bethesda, MD. 1993. <https://www.nlm.nih.gov/databases/download/hsdb.html>
 30. Final Report on the Safety Assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid. *Int J Toxicol*. 1987; 6: 321-401.
 31. Azcan N, Demirel E. Obtaining 2-octanol, 2-octanone, and sebacic acid from castor oil by microwave-induced alkali fusion. *Ind Eng Chem Res*. 2008; 47(6):1774-8.
 32. Vieira C, Fetzer S, Sauer SK, Evangelista S, Averbeck B, Kress M, Reeh PW, Cirillo R, Lippi A, Maggi CA, Manzini S. Pro- and anti-inflammatory actions of ricinoleic acid: similarities and differences with capsaicin. *N-S Arch Pharmacol*. 2001; 36: 87-95.
 33. Inoue A, Shoji A, Aso S. Allergic lipstick cheilitis due to ester gum and ricinoleic acid. *Contact Dermatitis*. 1998; 39: 39.
 34. Zhang QX, Wang QL, Han JH. Effect of fatty oil in *Periploca sepium* on neural system in mice. *Xi'an Med Univ*. 1995; 16:43-44.
 35. Foresti ML, Errazu A, Ferreira M. Effect of several reaction parameters in the solvent-free ethyl oleate synthesis using *Candida rugosa* lipase immobilised on polypropylene. *Biochem Eng J*. 2005; 25(1):69-77
 36. Hazarika S, Goswami N, Dutta N, Hazarika A. Ethyl oleate synthesis by porcine pancreatic lipase in organic solvent. *Chem Eng J*. 2002; 85(1):61-68