**Determination of Bioactive Components of Decholestrate, a polyherbal formulation by GC-MS Analysis**

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**Abstract:** In this study, the bioactive compounds of Decholestrate, a polyherbal formulation, have been evaluated using GC-MS technique. The chemical composition of the aqueous extract of decholestrate was investigated using Perkin-Elmer Gas Chromatography - Mass Spectroscopy. This analysis revealed the presence of Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (23.79), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- [S-(Z)]- (13.80), 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one (13.05). The compounds found in the study are reported to possess cardio toning properties and anti-hyperlipidemic activity. The result of this study offers a platform to reconfirm the properties of the components in decholestrate that are used as antihyperlipidemic agents.

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**Keywords:** GC-MS analysis; bioactivity of phytoconstituents; polyherbal formulation

**1. Introduction**

Plants serve as a basis of traditional medicinal systems for thousands of years (Hammer, 1999). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant (Koduru *et al.,* 2006). Plants produce a remarkable diverse array of over 5,00,000 low and high molecular mass natural products which are known as secondary metabolites (Fatope, 2001). Distinguished example of these compounds includes flavonoids, phenols, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi *et al*., 2008). It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Decholestrate is a polyherbal formulation that comprises of the extracts of the plant constituents from *Zingiber officinale* (Ginger)*, Tinospora cordifolia* (Guduchi)*, Piper longum* (long pepper), *Phyllanthus emblica* (Amla), *Embellia ribes* (False black pepper)*, Vigna unguiculata* (Cow pea)*, Garcinia cambogia* (Gambooge)*, Commiphora mukul* (Guggul)*, Camellia sinensis* (Tea leaves). The above herbs are reported to possess anti-diabetic, antihyperlipidemic (Al-Amin *et al.,* 2006), antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic (Singh *et al.,* 2003), antimicrobial (Ali *et al.,* 2007), antioxidant (Siddhuraju and Becker 2007), antiulcerogenic, hepatoprotective ([Krishnaveni](http://www.ncbi.nlm.nih.gov/pubmed?term=Krishnaveni%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20506691) and [Mirunalini](http://www.ncbi.nlm.nih.gov/pubmed?term=Mirunalini%20S%5BAuthor%5D&cauthor=true&cauthor_uid=20506691), 2010), antibacterial (Radhakrishnan *et al.,* 2011), anti-obesity ([Heymsfield](http://www.ncbi.nlm.nih.gov/pubmed?term=Heymsfield%20SB%5BAuthor%5D&cauthor=true&cauthor_uid=9820262) *et al.,* 1998), hypolipidemic (Wang *et al.,* 2004) and anti-carcinogenic properties (Hamilton-Miller, 2001) respectively. The aim of this study is to determine the organic compounds present in this formulation with the aid of GC-MS technique, which may provide an insight in its use as an antihyperlipidemic agent.

**2. Materials and Methods**

**Plant material**

Fresh plant/plant parts were purchased from local market in Coimbatore, Tamilnadu and Thrissur, Kerala, India. The taxonomic identities of these plants were confirmed and the voucher specimen numbers of the plants were deposited at Phytopharma testing lab, T. Stanes Company Ltd. Coimbatore. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

**Plant extraction procedure**

10 g of air-dried powder of the plant constituents was added to distilled water [1:10] and boiled on slow heat for 2 h with periodical stirring. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000rpm for 10 mins. The supernatant was collected and the above procedure was repeated thrice. After 6 hours, the supernatant collected at an interval of every 2 hours were pooled together and concentrated to make the final volume one-fourth of the original volume (Harbone, 1973). It was then autoclaved at 121o C and at 15 lbs pressure and stored at 4o C. The resulted powder was collected for every individual plant. These powders in equal proportions were mixed and homogenised in distilled water [1:20] at 60 o C to form a concoction. It was then vaccum dried and used for the GCMS analysis.

**Gas Chromatography – mass spectrum analysis (GC-MS)**

 GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions. Column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 μ Mdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; Helium (99.999%) was used as carrier gas at a constant flow of 1ml /min and an injection volume of 2 ml was employed (split ratio of 10:1); Injector temperature 250oC; Ion-source temperature 280oC. the oven temperature was programmed from 110oC (isothermal for 2 min) with an increase of 10oC / min, to 200oC then 5oC / min, to 280oC, ending with a 9 min Isothermal at 280oC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver5.2.0.

**Identification of components**

 Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, structure of the components in the test material was ascertained.

**3. Results**

Fourteen compounds were identified in Decholestrate formulation by GC-MS analysis. The active principle, Molecular weight (MW), Concentration (%), Molecular formula (MF), Retention Time (RT) and their bioactivity are presented in Figure 1, Table 1 and 2 dictates that the predominant compounds are Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (23.79), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (13.80), 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one (13.05), and Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]- (11.26).

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Figure 1. Chromatogram obtained from the GC-MS with the extract of Decholestrate

Table 1. Total ionic chromatogram (GC-MS) of decholestrate obtained with 70eV using an Elite -1 fused silica capillary column with He gas as the carrier.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **RT** | **Name of the compound** | **Molecular formula** | **MW** | **Peak Area%** |
| 1 | 12.39 | Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- | C15H24 | 258 | 23.79 |
| 2 | 12.75 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]- | C15H24 | 204 | 11.26 |
| 3 | 13.63 | 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane | C15H26O | 222 | 1.12 |
| 4 | 15.72 | 1-Iodo-2-methylnonane | C10H21I | 268 | 1.04 |
| 5 | 17.11 | 1-Iodo-2-methylundecane | C12H25I | 296 | 2.83 |
| 6 | 18.56 | Eicosane | C20H42 | 282 | 5.22 |
| 7 | 20.01 | Tridecane, 3-methyl- | C14H30 | 198 | 5.22 |
| 8 | 21.48 | Heptadecane, 2,6-dimethyl- | C19H40 | 268 | 4.47 |
| 9 | 22.94 | Decane, 2,3,5,8-tetramethyl- | C14H30 | 198 | 3.50 |
| 10 | 24.90 | 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one | C18H26O | 258 | 13.05 |
| 11 | 26.09 | Piperine | C17H19NO3 | 285 | 4.92 |
| 12 | 26.39 | 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl- | C15H26 | 206 | 3.28 |
| 13 | 32.17 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- | C15H26O | 222 | 13.80 |
| 14 | 33.01 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- | C15H26O | 222 | 6.49 |

Table 2. Activity of the components identified in Decholestrate [GC MS study]

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **No.** | **RT** | **Name of the compound** | **Molecular formula** | **MW** | **Peak Area %** | **Compound Nature** | **\*\*Activity** |
| 1 | 12.39 | Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- | C15H24 | 258 | 23.79 | Sesquiterpene compound | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |
| 2 | 12.75 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]- | C15H24 | 204 | 11.26 | Sesquiterpene compound | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |
| 3 | 13.63 | 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane | C15H26O | 222 | 1.12 | Sesquiterpene alcohol | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |
| 4 | 15.72 | 1-Iodo-2-methylnonane | C10H21I | 268 | 1.04 | Iodo compound | Antimicrobial |
| 5 | 17.11 | 1-Iodo-2-methylundecane | C12H25I | 296 | 2.83 | Iodo compound | Antimicrobial |
| 6 | 18.56 | Eicosane | C20H42 | 282 | 5.22 | Alkane | No activity reported |
| 7 | 20.01 | Tridecane, 3-methyl- | C14H30 | 198 | 5.22 | Alkane compound | No activity reported |
| 8 | 21.48 | Heptadecane, 2,6-dimethyl- | C19H40 | 268 | 4.47 | Alkane compound | No activity reported |
| 9 | 22.94 | Decane, 2,3,5,8-tetramethyl- | C14H30 | 198 | 3.50 | Alkane compound | No activity reported |
| 10 | 24.90 | 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one | C18H26O | 258 | 13.05 | Ketone compound | No activity reported |
| 11 | 26.09 | Piperine | C17H19NO3 | 285 | 4.92 | Alkaloid | [ATPase-Stimulant](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?ATPase-Stimulant) [Adrenergic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Adrenergic) [Analgesic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Analgesic) [Anesthetic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Anesthetic) [Antiaflatoxin](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiaflatoxin) [Antibacterial](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antibacterial); [Anticlastogen](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Anticlastogen) [Anticonvulsant](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Anticonvulsant) [Antiedemic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiedemic) [Antifertility](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antifertility) [Antiimplantation](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiimplantation) [Antiinflammatory](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiinflammatory) [Antileishmanic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antileishmanic) [Antimutagenic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antimutagenic) [Antinarcotic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antinarcotic); [Antioxidant](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antioxidant) [Antiplasmodial](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiplasmodial) [Antipyretic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antipyretic); [Antiseptic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antiseptic) [Antispasmodic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Antispasmodic) [Aryl-Hydrocarbon-Hydroxylase-Inhibitor](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Aryl-Hydrocarbon-Hydroxylase-Inhibitor)[CNS-Stimulant](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?CNS-Stimulant) Cardio tonic[Carminative](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Carminative); [Catecholaminogenic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Catecholaminogenic) [Diaphoretic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Diaphoretic) [Endorphinogenic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Endorphinogenic) [Epinephrininergic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Epinephrininergic) [FLavor](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?FLavor); [Hepatoprotective](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Hepatoprotective) [Insecticide](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Insecticide) [Parasiticide](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Parasiticide) [Pesticide](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Pesticide); [Respirostimulant](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Respirostimulant) [Secretogogue](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Secretogogue) [Serotoninergic](http://www.ars-grin.gov/cgi-bin/duke/chemical_activity.pl?Serotoninergic) |
| 12 | 26.39 | 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl- | C15H26 | 206 | 3.28 | Sesquiterpene compound | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |
| 13 | 32.17 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- | C15H26O | 222 | 13.80 | Sesquiterpene alcohol | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |
| 14 | 33.01 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- | C15H26O | 222 | 6.49 | Sesquiterpene alcohol | Antimicrobial, Anti-inflammatory, Anti hyperlipidemic |

**\*\*Activity source**: Dr Duke’s Phytochemical and Ethnobotanical data base.

**4. Discussions**

The results obtained show that majority of the predominant compounds in the formulation are found to be sesquiterpene compounds. Sesquiterpenes were reported to have anti-hyperlipidemic activity. The oxygen functional groups at the 3- and 8-positions and exo-methylene moiety in alpha-methylene-gamma-butyrolactone ring were found to be essential for the anti-hyperlipidemic activity of guaiane-type sesquiterpene (Shimoda *et al.,* 2003). Piperine, another component in the formulation, is a potent cardio toner. Thus based on these results it may be concluded that the components of decholestrate possess anti-hyperlipidemic activity. Further *in vivo* studies may be carried out to reconfirm the lipid lowering activity of the formulation.

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