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Phytochemical Characterisation Of *Cassia Montana* Endemic Medicinal Plant From Peninsular India

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Abstract: The genus Cassia has 38 species with five sub-species of Leguminosae (Caesalpiniaceae) possessed with appreciable medicinal taxon exerting a wide range of curing properties throughout the world. The present investigation focused on the phytochemical and pharmacological studies of *Senna montana* (Roth) V. Singh (*Cassia montana* Heyne ex Roth.) due to their remarkable medicinal properties. The leaf, stem and root bark extracts of *C. montana* reported here which have been known pharmacologically active components including Propanamide, 2-hydroxy, Benzene-methanamine, α 4-dimethyl-, α -methyl mannofuranoside, 2-Aminononadecan, α-Bisabolol , 2,3-Epoxyhexanol, D-glucopyranoside, methyl 3-O-methyl O, 5-O-methyl-D-gluconic acid, 1, 3-bis-t-butylperoxy-phthalan. The Bio-active compounds detected through GC-MS analysis of *C. montana* extracts are reported to possess biological activities like antitumor, anti-inflammatory anti- microbial, antioxidant, anticancer, anti-nociceptive, anti-arthritis, antioxidant, wound healing properties and skin problems. The extracts showed six constituents from the leaf, seven from stem bark, three from the root bark respectively. The preliminary phytochemical studies of these extracts resulted in about 12 from leaf, 12 from stem bark and 10 from root bark out of 12 groups of secondary metabolites respectively. The thorough literature survey revealed that, the present investigation was not reported and hence might be useful to the scientific community.

Keywords: *Cassia montana*, detection of secondary metabolites, phytochemical screening, biological effects

1. INTRODUCTION

Plants have the capacity of synthesizing a vast range of organic compounds through their metabolism, which are divided into primary and secondary metabolites [1]. Primary metabolites are universal in origin and involved in vital metabolic pathways, which include simple and complex compounds such as carbohydrates, proteins, and fats. Whereas the secondary metabolites are produced as bi-products like alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, saponins, glycosides are helpful in overcoming environmental stress as well as resistant to the various pests [2]. During the course of evolution plants adapted secondary biochemical pathways to synthesize a variety of chemicals in response to pathogens, pests, herbivores and competitors and to attract symbionts and pollinators besides medicaments[3]. Secondary metabolites found in plants are extremely important in medicine. As of right now, the World Health Organization (WHO) recognizes that over 11% of basic and necessary medications come from flowering plants. These substances, which are produced by plants for a variety of ecological uses, have shown impressive medicinal qualities and are still a vital source of the plant from pathogens. They are protective in function against UV radiation, free radicals and also act as anti-proliferative agents[5]. Plants possess a broad spectrum of secondary metabolites in different plant parts which exert biological activities[6]. Screening of active phytochemicals leads to the

invention of potential drugs which are used in innovative medications and therapies [4]. The antibiotic, antifungal and antiviral properties of secondary metabolites protect various diseases such as Diabetes, Arthritis, Cancer, Alzheimer's disease [7]. Medicinal plants are analyzed using high through-put analytical methods such as Gas Chromatography and mass spectroscopy (GC-MS) along with a chemical library, which became the most popular tool in the screening of medicinally useful plants. In this connection the present investigation on phytochemical evaluation of different parts of *Cassia montana* Heyne ex Roth (Family: Caesalpiniaceae), has been taken and reported for the first time. *C. montana* is an endemic plant of peninsular India [8].

The Genus *Cassia* is represented by approximately 500 species and has been separated into three genera *Cassia, Senna,* and *Chamae crista* and showed large species diversity with medicinal values. This genus consists of 38 species, 5 subspecies, 1 hybrid species, and 4 varieties which have been scientifically proved for wide range of pharmacological activities including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anti-ulcer, hypolipidemic, anti-atherosclerotic, and hepatoprotective properties have been reported from these species in modern medicine [9]. The chemical compounds derived from natural sources i.e., from plants, microbes and animals are defined as natural products, which possess biological/pharmacological activities [10]. These metabolites are synthesized by primary metabolism or rather secondary metabolism of living organisms. Secondary metabolites are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas [11]. *Cassia montana* leaf extract is reported to treat leucorrhoea in ethno-medicine [12]. *C. montana* is used in rheumatic pains [13]. *C. montana* contains Kaempferol, Quercetin, Kaempferol 3-O-rutinoside, Rutin C [14]. The antimicrobial activity and antioxidant effects of *C. montana* were reported [15]. However, the present investigation has focused on the preliminary phytochemical studies based on the chemical tests and characterization of leaf, stem bark and root bark alcoholic extracts using Gas Chromatography coupled with Mass Spectrophotometry. The components were identified using the database library as well as retention indices. Thorough review revealed that the present investigation has not been reported hitherto by the earlier researchers.

2. MATERIALS AND METHODS

2.1.Plant material

The leaves of *C. montana* were collected from Ardhagiri Hills of Chittoor District and the herbarium specimens were prepared as per the method of Jain and Rao (1983) [16]. and deposited in the Department of Botany, S.V. University Tirupati. Provisional identification of voucher specimen (MHL 314) was done by Prof. N. Yasodamma, Department of Botany, S. V. University, Tirupati with the help of Flora of the Presidency of Madras, Flora of Andhra Pradesh and Flora of Chittoor district [17, 18].. Identification is confirmed after comparing authentic specimens in Herbarium, Department of Botany, S. V. University, Tirupati.

2.2.Preparation of the extract

Leaf, stem bark, Root bark the selected three plants were collected and washed with water, cut into small fragments, shade dried and made into coarse powder which was sieved with 40 mesh sieves to get uniform particle size. A weighed quantity (50 g) of selected parts of the plant powder was weighed and immersed in 500 ml of distilled ethanol and water respectively for 24 h and the extracts were filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness in a water bath carefully at 50°C.

The extracts were stored in screw capped glass bottles in the refrigerator until further screening for preliminary Secondary metabolites. The extracts were used for Preliminary phytochemical screening of about ten groups of secondary metabolites, namely alkaloids, flavonoids, phenols, glycosides, tannins, steroids, lignins, saponins, terpenoids and anthocyanidins was done by the standard procedures prescribed by Gibbs (1974); Kokate *et al*. (2008), **[19,20].**

Five milligrams of each extract was taken and dissolved in methanol in a sterile clean test tube. The sample solution was filtered using 0.2 µm nylon membrane and the filtered sample solution was injected into the column for running GC-MS. The analysis was carried out on a Perkin-Elmer workstation, with model Clarus 680 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE). Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30m x 0.25 mm width film depth of 250 µm capillary tube was used under the following condition. The instrument has an oven with an initial temperature of 60 °C for

2 min and a ramp program which elevates from 10℃/min up to 300 ℃, further 6 min isothermal hold. Helium (He) carrier gas was used, with flow rate split ratios of 10:1. One µl of sample was injected and the temperature of the injector was maintained to 260 °C. The mass detector conditions were as follows. Transfer line temperature was 240 ℃ and ion source temperature was 240°C. The ionization mode electron impact was at 70 eV and a scan time of 0.2 sec with a scan interval of 0.1 sec. The fragments analyzed were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library. An individual component was recognized with typical mass spectra from National Institute of Standards and Technology (NIST- 2008) libraries which is inbuilt by the software of the GC-MS system (TurboMassver 5.4.2) and literature data. The individual phytochemicals present in the crude extract were separated by the gas chromatography column. An individual compound separated by GC enters the Mass Spectrum (MS) and gets ionized. The MS ionizing spectrum was recorded and compared to the MS spectrum of known compounds in the NIST library. Each compound was compared with a percentage score of reverse and forward spectrum. The MS spectrum displays the molecular weight of individual molecules accurately.

3. RESULTS AND DISCUSSION

3.1.**RESULTS**

The preliminary screening of secondary metabolites revealed the presence of 10 to 12 phytoconstituents in all the selected plantextracts as Alkaloids, steroids, lignins, flavonoids, phenols, terpenoids, tannins, anthocynidins, cardiac glycosides and saponins reducing sugars (Table 1). GC-MS of *C. montana* leaf extract resulted 10 compounds, Propanamide,2-hydroxy, 5-O-methyl-d-gluconic acid, diethylamide, Benzenemethanamine, α-.,4-dimethyl-, α-methylmannofuranoside, D-glucopyranoside,methyl, 3-O-methyl, 5-O- methyl-d-gluconic acid, diethylamide and 1,3-bis-t-butylperoxy-phthalan (Table 2, Fig.1). GC-MS chromatogram detected 7 peaks in *C. montana* stem bark extract (Fig.2 and 4) like 2-Amininonadecan, α-Bisabolol, Cyclopropyl Carbinol, 2,3-Epoxyhexanol, 1- Octanamine, N-Methyl-N-Nitroso, 3-Methyl Mannoside, Benzaldehyde, 2-Nitro-Diaminomethylidene Hydrazone (Table 3). GC-MS chromatogram of *C. montana* root bark extract has showed 3 peaks (Fig.3 and 6) representing, α-methyl mannofuranoside, Benzenemethanamine, α,4- dimethyl-, α-methylmannofuranoside and Propanamide 2-hydroxy (Table 4) among which *α*-methyl mannofuranoside is present in all three tested extracts showing highest peak area representing more than 82%.

3.2.**DISCUSSION**

Propanamide, 2- hydroxy is used in the topical application of diseases such as dry skin, eczema, and plantar hyperkeratosis, dandruff, acne and warts [21]. Benzenemethanamine, α-4-dimethyl- acts as 5-α-reductase inhibitor, α-Amylase inhibitor, α-Glucosidase inhibitor, TNF-α-inhibitor. α-methyl mannofuranoside is Catechol- O-Methyltransferase inhibitor, 5-α –Reductase inhibitor, α -Amylase inhibitor, α- Glucosidase inhibitor, TNF- α- inhibitor. 2-Amininonadecan is Phosphodiesterase-3 inhibitor and anesthetic. α-Bisabolol is a small, plant derived, oily sesquiterpene alcohol with anti-inflammatory, anti-microbial, antioxidant, antitumor, cardioprotective, antinociceptive and wound healing properties[22-30]. 2,3-Epoxyhexanol shows anaphylactic, antitumor, GABA-nergic, increase Natural Killer cell Activity, inhibit production of TNF, Myo-neuro stimulant, N-Cholinolytic, NADH-oxidase-inhibitor, NADH-Ubiquinone-Oxidoreductase inhibitor, Stimulate Norepinephrine Production. 3-Methyl Mannoside has Antitumor (nasopharynx), gaba-nergic, Increase Natural killer (NK) Cell Activity, Myo-neuro-stimulant, NADH ubiquinone-Oxidoreductase-inhibitor, Narcotic Properties. Catechol-O-Methyltransferase-inhibitor, D-glucopyranoside, methyl 3-O-methyl-O, decreases oxalate excretion, increases Vit D Bioavailability and possesses antitumor (Ovary), anticancer (Oral), demulcent, TNF- α-inhibitor, diuretic properties. 5-O-methyl-D-gluconic acid dimethyl amide acts as anticancer, antidote, blood thinning properties and inhibits the production of TNF. 1, 3-bis-t-butylperoxy-phthalan inhibits the production of TNF and acts as anticancer, antidote, blood thinner, demulcent. These compounds reduce inflammation in various autoimmune diseases such as rheumatoid arthritis, psoriasis and ankylosing spondylitis, beneficial in treating Benign prostatic hypertrophy, cancer (Oral), antitumor (Ovary), polycystic ovary syndrome, diabetes, kidney stones, bacterial and fungal infections [31].

4. CONCLUSION

Bio-active compounds detected through GC-MS analysis of *C. montana* extracts are reported to possess biological activities like antitumor, anti-inflammatory anti-microbial, antioxidant, anticancer, antinociceptive, antiarthritic, antioxidant, wound healing properties and skin problems. Moreover, the leaf extracts reported for potential antimicrobial activity [32] has been supported the traditional claims and the phytochemical composition revealed that the potential diversity of secondary metabolites and groups which were paying attention for the further studies to confirm the medicinal properties. However, the pharmacological effects of chemical constituents adopted based on the available literature are yet to confirm using the extracts of *C. montana* on *in vivo* systems and further studies required to utilize as effective medicaments.

Table 1: Phytochemical groups of *C. montana*

S. No	Test	Leaf	Stem Bark	Root Bark
1.	Alkaloids	++	+	+
2.	Flavonoids	+	+	+
3.	Phenols	+	+	+
4.	Terpenoids	++	+	+
5.	Steroids	+	+	+
6.	Anthocyanidins	+	+	+
7.	Anthraquinones	+	+	-
8.	Saponins	+	+	+
9.	Tannins	+	+	+
10.	Lignins	+	+	+
11.	Glycosides	+	+	-
12.	Reducing sugars	+	+	+
	Total	12	12	10

Table 2: Chemical constituents of *C. montana* leaf extract analyzed through GC-MS studies

S No	RT	Name of the compoumd	Biological Activity	Height	Area %
1	4.119	Propanamide 2-Hydroxy	17-β-hydroxy steroid dehydrogenase-inhibitor, Testosterone-Hydroxylase-inducer.topical application of diseases such as dry skin, eczema, and plantar hyperkeratosis, dandruff, acne and warts	2,877,770	2.05
2	18.620	Benzenemethanamine,. α,4-dimethyl	5-α-Reductase-inhibitor, α- Amylase- inhibitor, α-Glucosidase-Inhibitor, TNF- α - Inhibitor.	2,101,005	2.01
3.	21.896	α-methyl mannofuranoside	5-α-Reductase-inhibitor, α-Amylase- ihibitor, α-Glucosidase-Inhibitor, Catechol-O-methyl transferase-inhibitor	14,532,834	84
4.	22.846	D-glucopyranoside, methyl 3-O-methylo	Decrease oxalate excretion, anticancer (Oral), Increase Vit D bioavailability, antitumor (Ovary), Demulcent, TNF-α-Inhibitor, Food dye,	6,438,618	7.4
5	23.587	5-O-methyl-d-gluconic acid dimethylamide	Anticancer, antidote, increase osteocalcin, ovulent, oxytocic, demulcent, diuretic	2,678,407	2.45

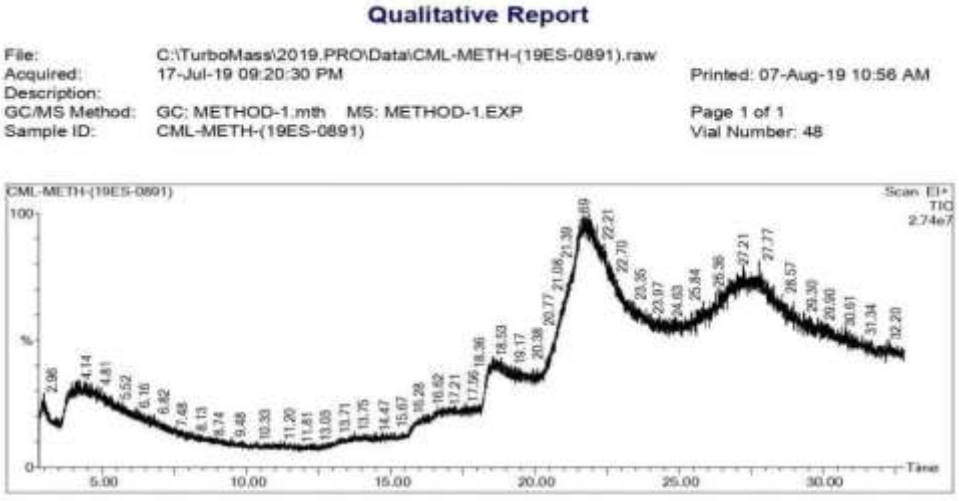
Table 3: Chemical constituents of *C. montana* stem bark extract analyzed through GC-MS

S No	RT	Name of the compoumd	Medicinal Uses	Height	Area %
1	2.518	Cyclopropyl Carbinol	Phosphodiesterase 3 inhibitor, anesthetic	1,430,955.5	4.173
2	13.463	2,3-Epoxyhexanol	Anaphyletic, Antitumor, GABA-nergic, Increase Natural Killer cell Activity, Inhibit production of TNF, Myo-neuro_stimulant, N- Cholinolytic, NADH-oxidase-inhibitor, NADH- Ubiquinone-oxidoreductase-inhibitor, stimulate Norepinephrine Production	1,088,593.1	3.174
3	13.953	1-Octanamine, N-Methyl-N-Nitroson	Antitumor (nasopharynx), gaba-nergic, Increase Natural killer (NK) Cell Activity, Myo-neuro-stimulant, NADH biquinone-Oxidoreductase-inhibitor, Narcotic	615,868.4	1.796
4	16.634	3-Methylmannoside	5-Alpha-Reductase-Inhibitor, Alpha-Amylase- Inhibitor, Alpha-Glucosidase-Inhibitor, TNF-alpha-Inhibitor. Catechol-O-Methyl-Transferase-Inhibitor	28,362,458.0	82.705
5.	24.512	α –Bisabolol Sesquiterpene	antiinflammatory and anti-microbial, Antioxidant, antitumor, cardioprotective, antinociceptive, wound healing properties.	2,795,540.0	8.152

Table 4: Chemical constituents of *C. montana* methanolic root bark extract analyzed through GCMS

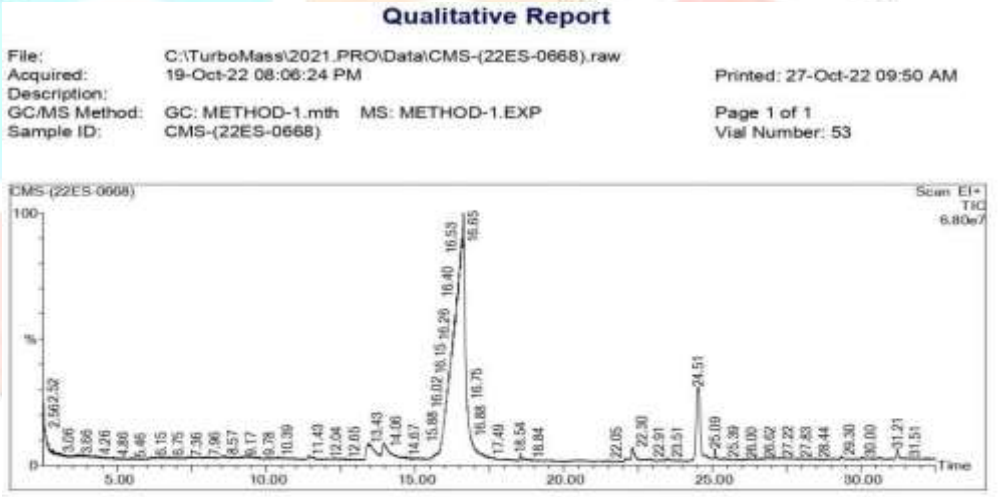
S No	**RT**	**Name of the compound**	**Biological Activity**	**Height**	**Area %**
1	2.518	Propanamide 2-Hydroxy	17-β-hydroxysteroid dehydrogenase-Inhibitor	17,826,972	3.79
2	13.463	Benzenemethanamine, *α*,4- dimethyl-	5-*α*-Reductase-Inhibitor, *α*-Amylase-Inhibitor, *α*-Glucosidase-Inhibitor, TNF-*α*-Inhibitor.	8,547,998	4.12
3	16.924	*α*-methyl mannofuranoside	5-*α*-Reductase-Inhibitor, *α*-Amylase- Inhibitor, *α*-Glucosidase-Inhibitor, TNF-*α*-Inhibitor. Catechol-O-Methyl-Transferase-Inhibitor	94,710,368	100

Fig. 1. GCMS Chromatogram of *C. montana* methanolic leaf extract



#	RT	Scan	Height	Area	Area %	Norm %
1	21.696	3818	14,532,834	19,010,820.0	83.953	100.00
2	22.846	4008	6,438,618	2,583,604.2	11.409	13.59
3	23.587	4156	2,678,407	555,220.7	2.452	2.92
4	26.718	4782	3,159,706	495,077.5	2.186	2.60

Fig 2. GCMS Chromatogram of *C. montana* methanolic stem bark extract



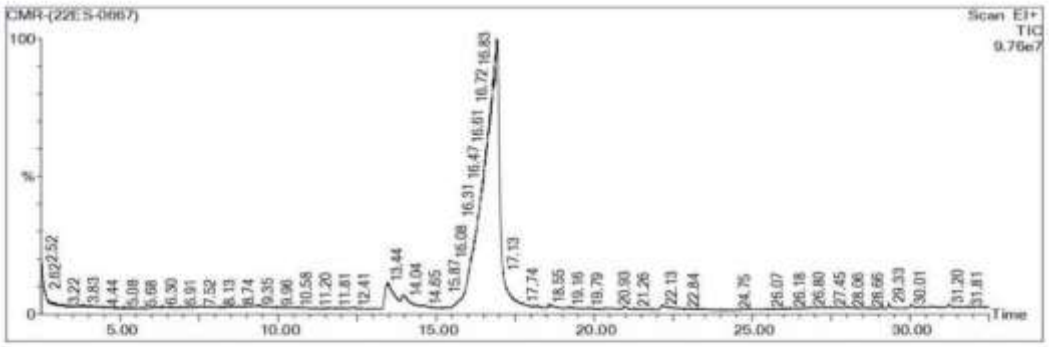
#	RT	Scan	Height	Area	Area %	Norm %
1	2.518	4	16,583,676	1,430,955.5	4.173	5.05
2	13.463	2192	4,026,592	1,088,593.1	3.174	3.84
3	13.953	2260	3,297,643	615,868.4	1.796	2.17
4	16.634	2826	65,204,384	28,362,458.0	82.705	100.00
5	24.512	4401	19,204,176	2,795,540.0	8.152	9.86

Fig 3. GCMS Chromatogram of *C. montana* methanolic root bark extract

Qualitative Report

File: C:\TurboMass\2021.PRO\Data\CMR-(22ES-0667).raw
Acquired: 19-Oct-22 07:28:49 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
Sample ID: CMR-(22ES-0667)

Printed: 27-Oct-22 09:48 AM
Page 1 of 1
Vial Number: 52



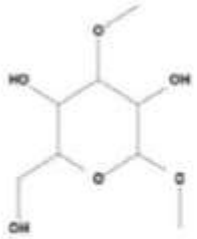
#	RT	Scan	Height	Area	Area %	Norm %
1	2.518	4	17,826,972	2,088,273.6	3.514	3.79
2	13.463	2192	8,547,998	2,268,364.8	3.817	4.12
3	16.924	2884	94,710,368	55,063,704.0	92.668	100.00

Fig. 4 Chemical constituents of *C. montana* leaves

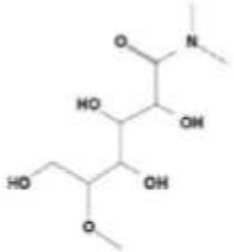
PROPANAMIDE, 2-HYDROXY

. α.-METHYL MANNOFURANOSIDE

D-GLUCOPYRANOSIDE, METHYL 3-O-METHYL



5-O-METHYL-D-GLUCONIC ACID DIMETHYLAMIDE



1,3-BIS-T-BUTYLPEROXY-PHTHALAN

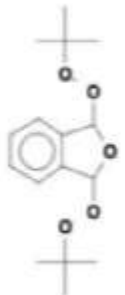


Fig. 5 Chemical constituents of *C. montana* from Stem bark

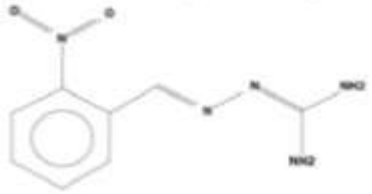
CYCLOPROPYL CARBINOL

2, 3-EPOXYHEXANOL

1-OCTANAMINE, N-METHYL-N-NITROSO

ETHYLMANNOSIDE

ENZALDEHYDE, 2-NITRO-, DIAMINOMETHYLIDENHYDRAZONE

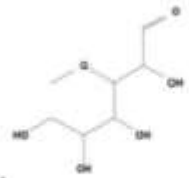


ALPHA.-BISABOLOL

2-AMINONONADECANE

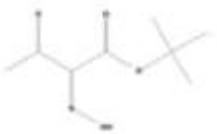
Fig. 6 Chemical constituents of *C. montana* from root bark

3-O-METHYL-D-GLUCOSE

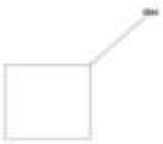


1-OCTANAMINE, N-METHYL-N-NITROSO

(Z)-2-HYDROXYIMINO-3-OXOBUTYRIC ACID, 1,1-DIMETHYLETHYL ESTER



CYCLOBUTANOL



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