# CHARACTERIZATION OF CHEMICAL COMPOUNDS OF MEDICINAL IMPORTANCE IN *DIOSPYROS MONTANA* ROXB.

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**ABSTRACT**

Plants and their constituents are precious gift of nature to the humans being. They fulfill various routine requirements of human being including the most important health care issues. The aim of the present study is to investigate the bioactive compounds in leaf, stem and seeds of *Diospyros montana* Roxb. using aqueous, ethanol, acetone and hexane as solvents. A preliminary phytochemical analysis has revealed the presence of alkaloids, flavonoids, glycosides, phenols, quinones, steroids and terpenoids in all the prepared extracts. Maximum number of phytochemicals was reported in ethanol leaf extract. The GC-MS analysis of ethanol extract of leaves has yielded 42 bioactive compounds. Out of these, 28 compounds possess a wide range of pharmacological activities. Camphor, (+)-2-bornanone, phytol, acetate, 1-monolinoleoylglycerol trimethylsilyl ether, oxirane, [(hexadecyloxy) methyl]-, oleic acid and 3,7,11,15-tetramethyl-2-hexadecen-1-ol are amongst several compounds of medicinal interest. Fourier transform infrared (FT-IR) spectroscopy has provided the information related to the functional groups. The presence of a variety of chemical compounds in this species, particularly in leaves, has supported its use in the traditional health care formulations. The information generated during the present investigation may provide tools for designing new or alternative medicinal products.

**Keywords**: Phytoconstituents, Leaf, Stem, Seed, Medicinal value, FT-IR, GC-MS analysis.

## INTRODUCTION

Plants are known to produce a variety of primary and secondary metabolites in response to various biotic and abiotic stresses. These metabolites have the potential to cure number of human as well as animal diseases[1]. Secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, tannins, terpenoids etc. possess antioxidant, anti-inflammatory, antitumor, anti-carcinogenic and antimicrobial activities[2]. These medicinal and nutritional properties in the form of food items have linked the plant species with the health benefits[3].

*Diospyros montana* Roxb. a member of family Ebenaceae, is commonly known as Bistendu, Kendu, Bombay ebony and Kala dhao. Family Ebenaceae, contains around 500 species widely distributed in the tropics and subtropics[4,5]. The World Checklist of Selected Plant Families and World Flora Online has recognized around 755 accepted taxa[6,7]. The genus *Diospyros* is comprised of 240 species, including deciduous, evergreen trees, shrubs and small bushes. Out of the 240 species, 59 occur in India[8,9].

*D. montana Roxb.* is a deciduous small tree which may attain height up to 10-12 m. Stem bark is smooth, grey to yellowish, young shoots are glabrous to pubescent, stiff branches and spiny trunk. Leaves are simple, alternate, softly pubescent, ovate, ovate-elliptic, lance-shaped, entire, somewhat cordate base and obtuse apex. Leaf surface is smooth adaxial and velvety on the abaxial. Flowers are unisexual, 2-6 axillary umbels, creamy white or greenish, calyx tubular with 4 lobes, corolla urceolate, imbricate which curved back, stamens connate at base and glabrous ovary. Fruit is berry, spherical, green and yellow-brown when ripened. Fruiting calyx encircles the fruit. It contains 3-6 rough and black seeds[10].

Ayurveda and Unani systems of medicine have highlighted the significance of genus *Diospyros*. Bark of this plant is used by natives for the treatment of depression, jaundice, vomiting and roots as abortifacient. Different plant parts have been utilized in indigenous medicines for the treatment of delirium, fever, diarrhoea and pneumonia[4]. *D. montana* possesses antihelmithic, anticancer, anti-

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inflammatory, antimalarial, antiviral and antipyretic properties[11,12]. Compounds like bis-naphthoquinone and diospyrin isolated from *D. montana* were reported to be active against protozoan parasites and carcinoma in mice[11,13]. The plant has also been used in the treatment of cough, ulcer, anti-hypersensitive and snake bites[14,15].

Keeping in view the significance of *D. montana* in traditional medicine, present study has been planned to look into the detailed account of chemical compounds through phytochemicals, FT-IR and GC-MS analysis.

## MATERIALS AND METHODS

An expedition was made in the study area (district Rupnagar, Punjab) for documentation and collection of the research materials. *D. montana* has been widely growing in various sites along the road, barren land and hilly area in the village Spalwan (31°16'0.98"N 76°20'16.10"E) in Rupnagar, Punjab. Photography of plant species was done in natural habitat (Fig. 1). The plant specimens were identified using the available literature, flora and by consulting the Herbarium, Department of Botany, Panjab University, Chandigarh[16,17].

### Collection of plant material

Different plant parts (leaves, stem and seeds) were collected for investigation. The material was cleaned under running tap water and then with distilled water. The plant material was allowed to shade dry at room temperature and powdered using an electric blender. The powdered material was stored in air tight bags for further use.

### Preparation of aqueous extract

20 g plant powder was added to 100 mL distilled water taken in a conical flask. The flask was kept on a rotary shaker for 24 h[18]. After this, the mixture was filtered through muslin cloth followed by Whatman filter No. 1. The obtained extract was stored in airtight vials at 4 °C for further studies.

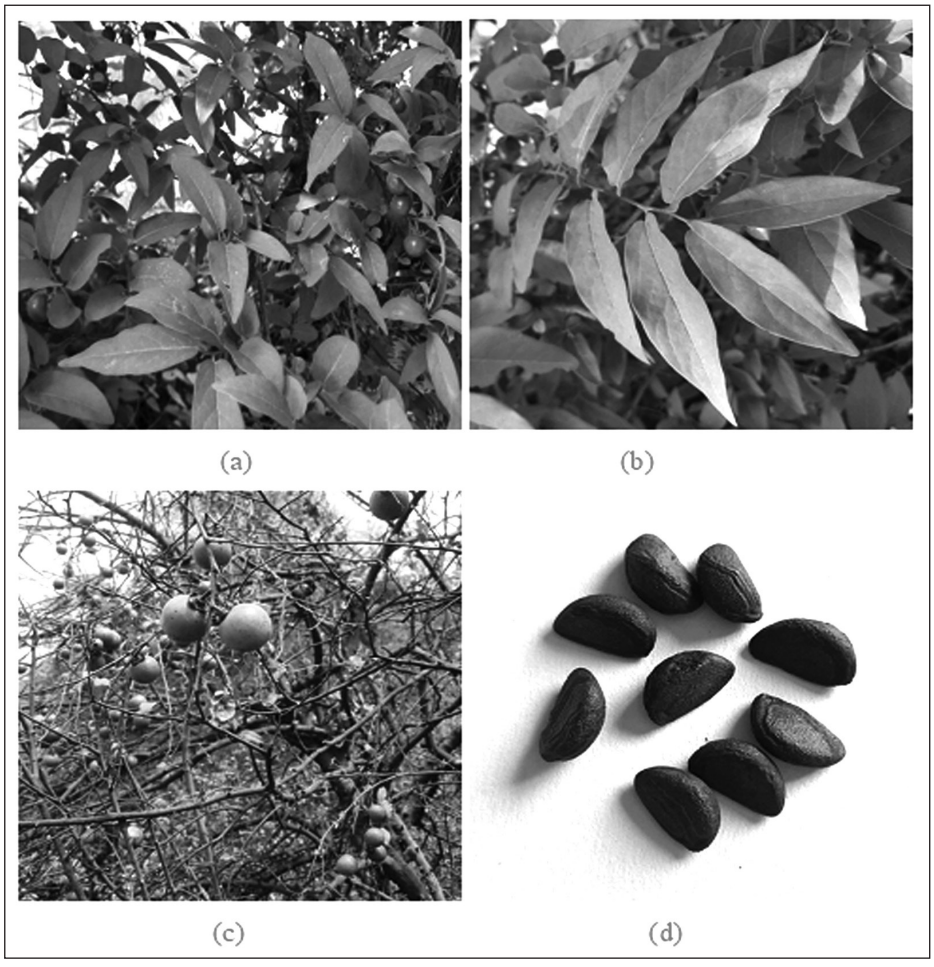


**Fig. 1 (a-d): *D. montana* (a) Whole plant (b) Leaves (c) Plant bearing fruits (d) Seeds**

### Preparation of ethanol, acetone and hexane extracts

The plant powder 10 g was extracted through the Soxhlet apparatus in 130 mL of ethanol at 60 °C for 24 h[19]. The obtained extract was evaporated to 1/3 of its original volume at room temperature and stored in airtight vials at 4 °C for further use. The preparation of extracts in acetone and hexane is similar to that in ethanol, except that temperature is adjusted according to the boiling points of the respective solvent.

### Phytochemical screening

Aqueous, ethanol, acetone and hexane extracts of leaves, stem and seeds of *D. montana* were screened for the presence of different phytoconstituents such as alkaloids, flavonoids, carbohydrates, glycosides, gum and mucilage, phenolics, proteins, resins, saponins, steroids, terpenoids etc. The analysis was carried out using standard procedures[20-27].

**Table I: Phytochemical account of *D. montana* (Stem, leaves and seeds)**

| Phytochemical test | Stem | | | | Leaves | | | | Seeds | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aq | Eth | Ace | Hex | Aq | Eth | Ace | Hex | Aq | Eth | Ace | Hex |
| Alkaloids (Mayer's reagent test) | + | ++ | + | - | + | ++ | + | - | ++ | + | + | - |
| Amino acid | - | - | - | - | - | - | - | - | - | - | - | - |
| Anthocyanin | + | + | + | - | + | + | ± | - | + | + | + | + |
| Anthraquinones | + | + | ± | - | ± | ++ | ± | - | ± | ± | + | - |
| Betaxanthin | + | + | + | ± | + | + | ± | ± | - | ± | - | - |
| Carbohydrates (Molisch test) | + | ++ | ++ | + | + | ++ | + | ++ | + | ++ | + | + |
| Cardiac glycosides | ++ | ++ | ++ | + | + | ++ | ++ | + | + | + | + | + |
| Coumarin | ± | - | - | - | ± | + | - | - | - | - | - | - |
| Diterpenoids | + | + | - | - | ± | + | + | + | - | - | - | - |
| Flavonoids NaOH test | ± | - | - | - | + | ± | - | ± | - | - | - | - |
| $H_2SO_4$ test | + | + | + | + | + | + | - | - | + | + | + | + |
| Glycosides | + | ++ | ++ | + | + | +++ | ++ | + | ++ | ++ | ++ | ++ |
| Gums and mucilage | + | - | - | - | ++ | - | - | - | ++ | - | - | - |
| Oxalate | - | - | - | - | + | ++ | + | - | ± | - | ± | - |
| Phenol | ± | + | + | - | ++ | + | + | - | ± | + | + | - |
| Phlobatannins | ± | - | ± | - | + | - | - | - | - | ± | ± | - |
| Proteins (Xanthoproteic test) | + | ++ | + | ± | ++ | + | + | + | ++ | ++ | ++ | ± |
| Quinones | + | + | + | ± | + | + | + | + | ± | ++ | + | ± |
| Reducing sugar | ++ | + | ++ | - | + | + | + | ± | ++ | + | ++ | - |
| Resin | + | + | + | ± | + | ± | + | - | + | ± | - | - |
| Saponins (Froth test) | ± | - | - | - | ++ | - | - | - | + | - | - | - |
| Starch | ++ | + | + | + | + | +++ | ++ | + | ++ | + | ++ | ± |
| Steroids ($H_2SO_4$ test) | + | + | + | + | + | + | + | + | + | + | + | + |
| Tannin $FeCl_3$ test | ± | + | + | - | ++ | + | + | - | ± | ± | + | - |
| KOH test | ± | + | + | - | ± | + | + | - | ± | - | - | - |
| Terpenoids ($CHCl_3$ test) | + | ++ | ++ | + | ++ | +++ | ++ | + | ++ | ++ | + | + |

*The symbols: - (absent), ± (present in traces), + (present), ++ (moderately present) and +++ (present in abundance). Aq. (Aqueous), Eth. (Ethanol), Ace. (Acetone) and Hex. (Hexane)*

## Gas chromatography-Mass spectroscopy (GC-MS) analysis

GC-MS analysis of the ethanol extract of leaves of *D. montana* was performed using Thermo Trace 1300 GC with Thermo TSQ 8000 Triple Quadrupole MS at CIL/SAIF, Panjab University, Chandigarh. Injector temperature and injector volume was 250 °C and 1.00 µL, relatively with 32.10 run time. TG5MS (30×0.25 mm, 0.25 µm) column composed of 5 % diphenyl and 95 % dimethyl polysiloxane column was used for the analysis. Helium acted as carrier gas at 1.5 mL $min^{-1}$ constant flow with 45 mL $min^{-1}$ split flow because in GC, extract separated through heating

**Table II: Detailed account of the compounds studied in ethanol extract of *D. montana* leaves**

| PA | Compound | RT | Area (%) | MF | MW | Bioactivity | References |
|---|---|---|---|---|---|---|---|
| 1. | Oxalic acid | 3.39 | 6.14 | $C_2H_2O_4$ | 90.03 | Calcium regulation, Detoxification | 45 |
| | Hydrazine, methyl- | 3.39 | 6.14 | $CH_6N_2$ | 46.07 | Antineoplastic | 46 |
| 2. | 3,5-Dithiahexanol 5,5-dioxide | 6.03 | 2.49 | $C_4H_{10}O_3S_2$ | 170 | Antitumour | 47 |
| 3. | Methyltartronic acid | 6.80 | 0.74 | $C_4H_6O_5$ | 134 | Cytotoxic, Antibacterial | 48,49 |
| 4. | Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-, (1S)- | 8.04 | 2.35 | $C_{10}H_{16}O$ | 167 | Antimicrobial | 50,51 |
| | (+)-2-Bornanone | 8.04 | 2.35 | $C_{10}H_{16}O$ | 152 | Antitumor, Anticancer, Antimicrobial, Analgesic, Antibacterial, Antioxidant, Sedative, Anti-inflammatory | 52,53 |
| | Camphor | 8.04 | 2.35 | $C_{10}H_{16}O$ | 152 | Rheumatism, Antimicrobial Sprains, Antiasthma, Ingestion, Anticancer, Brain dysfunctions, Anti-mutagenic | 54,55,56 |
| | Cyclohexanone, 2-methyl -5-(1methylethenyl)-, trans | 8.04 | 2.35 | $C_{10}H_{16}O$ | 152 | Antibacterial, Antioxidant | 57 |
| 5. | Tridecane | 8.75 | 2.53 | $C_{13}H_{28}$ | 184 | Neuroleptic, Emollient, Anticancer, Antimicrobial | 38 |
| | Undecane | 8.75 | 2.53 | $C_{11}H_{24}$ | 156 | Antiallergic, Antibacterial, Anti-inflammatory | 58,43 |
| | Dodecane | 8.75 | 2.53 | $C_{12}H_{26}$ | 170 | Neurotropic, Antineoplastic, Phobic disorders treatment, Tetany, Pulmonary edema, Antimicrobial, Antioxidant, Antifungal | 59,60 |
| 6. | Cyclohexane, (4-methyl pentyl)- | 9.39 | 0.39 | $C_{12}H_{24}$ | 168 | Antimicrobial, Antidiabetic, Sedative | 41 |
| 7. | 5-Eicosene, (E)- | 11.13 | 0.87 | $C_{20}H_{40}$ | 280 | Antimicrobial, Antibacterial, Antioxidant | 61,62 |
| 8. | 3-Tetradecene, (Z)- | 11.42 | 1.14 | $C_{14}H_{28}$ | 196 | Antibacterial, Neutrophilic, Antiasthmatic | 61,63 |
| | 9-Octadecene, (E)- | 11.42 | 1.14 | $C_{18}H_{36}$ | 252 | Antioxidant, Antimicrobial | 64 |
| 9. | Cyclohexane, octyl- | 12.22 | 0.34 | $C_{14}H_{28}$ | 196 | Antimicrobial | 65 |
| 10. | Diethyl phthalate | 13.89 | 17.85 | $C_{12}H_{14}O_4$ | 222 | Anti-androgenic, Antimicrobial | 66,67 |
| | 5-Anilino-1,2,3,-thiadiazol | 13.89 | 17.85 | $C_8H_7N_3S$ | 178 | Antileukemic, Antioxidant | 68,69 |
| 11. | Cyclooctasiloxane, hexadecamethyl- | 14.40 | 3.39 | $C_{16}H_{48}O_8Si_8$ | 593 | Antimicrobial | 70 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12. | Phytol | 15.07 | 0.79 | $C_{20}H_{40}O$ | 296 | Anticonvulsant, Antidiabetic, Antimicrobial, Anticancer, Anti-inflammatory, Immunoadjuvant, Diuretic, Antifungal, Antimalarial | 71,72,73,42 |
| 13. | Octahydrobenzo[b] pyran, 4a-acetoxy-5,5,8a-trimethyl | 15.25 | 0.52 | $C_{14}H_{24}O_3$ | 240 | Anti-inflammatory, Antifungal | 74 |
| 14. | 3-Eicosene, *(E)*- | 16.21 | 1.33 | $C_{20}H_{40}$ | 280 | Antibacterial | 75 |
| | 3-Octadecene, *(E)*- | 16.21 | 1.33 | $C_{18}H_{36}$ | 252 | Antibacterial, Antioxidant, Anticancer | 76 |
| | 10-Heneicosene (c,t) | 16.21 | 1.33 | $C_{21}H_{42}$ | 294 | Antineoplastic | 77 |
| 15. | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | 16.69 | 0.95 | $C_{20}H_{40}O$ | 296 | Anticancer, Antidiuretic, Antimicrobial, Antioxidant, Anti-inflammatory | 78,79 |
| | Phytol, acetate | 16.69 | 0.95 | $C_{22}H_{42}O_2$ | 338 | Anti-inflammatory, Diuretic, Antitrypanosomal, Antimicrobial, Anticancer, Antileishmanial | 74,80 |
| | Oxirane, hexadecyl- | 16.69 | 0.95 | $C_{18}H_{36}O$ | 268 | Antibacterial, Antimicrobial, Antioxidant, Antipyretic, Anti-inflammatory, Analgesic | 81 |
| | E-6-Octadecen-1-ol acetate | 16.69 | 0.95 | $C_{20}H_{38}O_2$ | 310 | Antimicrobial | 82 |
| 16. | Galaxolide 2 | 16.85 | 17.45 | $C_{18}H_{26}O$ | 258 | Antimicrobial | 83 |
| 17. | Butyrolactone | 17.30 | 3.61 | $C_4H_6O_2$ | 86.0 | Hallucinogenic drug, Anxiolytic, Antimicrobial | 84,85 |
| 18. | n-Hexadecanoic acid | 18.07 | 1.46 | $C_{16}H_{32}O_2$ | 256 | Antioxidant, Antiandrogenic, Hemolytic, Anti-inflammatory, Antibacterial, Antioxidant, Antifungal, Antirheumatic | 71,86,87 |
| | L-(+)-Ascorbic acid 2,6-dihexadecanoate | 18.07 | 1.46 | $C_{38}H_{68}O_8$ | 652.9 | Antimetastatic, Anticancer, Antioxidant, Anti-inflammatory, Antibacterial, Enhance sperm motility | 88,89 |
| | Eicosanoic acid | 18.07 | 1.46 | $C_{20}H_{40}O_2$ | 312 | Anticancer, Anti-inflammatory, Antifungal | 90 |
| | Tridecanoic acid | 18.07 | 1.46 | $C_{13}H_{26}O_2$ | 214 | Anticancer, Antioxidant, Anti-inflammatory, Cardioprotective | 91 |
| 19. | Hexadecanoic acid, ethyl ester | 18.24 | 3.26 | $C_{18}H_{36}O_2$ | 284 | Antioxidant, Hemolytic, Antioxidant, Antiandrogenic, Hypocholesterolemic | 71,92 |
| 20. | Benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester | 19.04 | 0.40 | $C_{16}H_{30}O_4Si_3$ | 370 | Antimicrobial | 70 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21. | 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl- | 19.38 | 1.44 | $C_{20}H_{40}O$ | 296 | Antifungal, Antiviral, Anti-inflammatory | 93 |
| 22. | cis-Vaccenic acid | 19.87 | 1.96 | $C_{18}H_{34}O_2$ | 282 | Antiasthmatic, Anti-inflammatory, Anticancer, Antibacterial | 94, 95, 96 |
| | Oleic Acid | 19.87 | 1.96 | $C_{18}H_{34}O_2$ | 282 | Anticancer, Antioxidant, Antitumor | 97,98 |
| | trans-13-Octadecenoic acid | 19.87 | 1.96 | $C_{18}H_{34}O_2$ | 282 | Anti-inflammatory, Anticancer | 40 |
| 23. | E-9-Octadecenoic acid, ethyl ester | 20.12 | 1.34 | $C_{20}H_{38}O_2$ | 310 | Antioxidant, Anti-inflammatory, Muscle weakness, Pulmonary edema, Anemia, Diarrhea | 60,99 |
| | Eicosanoic acid, phenylmethyl ester | 20.12 | 1.34 | $C_{27}H_{46}O_2$ | 402 | Anti-inflammatory | 100 |
| 24. | 9,12,15-Octadecatrienoic acid,2-[(trimethylsilyl) oxy]-1[[ (trimethylsilyl) oxy]methyl] ethyl ester, (Z,Z,Z)- | 20.32 | 0.45 | $C_{27}H_{52}O_4\ Si_2$ | 496 | Antioxidant, Antidiabetic, Anti-inflammatory, Anticancer, Antihistaminic Antioxidant, Antiarthritis | 101,102,103 |
| | 1-Monolinoleoyl Glycerol trimethylsilyl ether | 20.32 | 0.45 | $C_{27}H_{54}O_4Si_2$ | 498 | Antimicrobial, Antidiabetic, Antiarthritis, Antioxidant, Diuretic, Anti-inflammatory, Antiasthmatic, Antidiabetic, Hepatoprotective | 71,104,105 |
| 25. | Tetracosamethyl-cyclododecasiloxane | 21.75 | 0.31 | $C_{24}H_{72}O_{12}Si_{12}$ | 889 | Hepato-protective, Appetizer Antispasmodic, Antirheumatic | 105 |
| 26. | Heptasiloxane, hexadecamethyl- | 23.64 | 1.07 | $C_{16}H_{48}O_6Si_7$ | 533 | Antimicrobial | 70 |
| | Hexasiloxane, tetradecamethyl | 23.64 | 1.07 | $C_{14}H_{42}O_5Si_6$ | 458 | Antiandrogenic, Anticoranary, Antieczemic | 106 |
| 27. | Oxirane, [(hexadecyloxy) methyl]- | 23.86 | 0.54 | $C_{19}H_{38}O_2$ | 298 | Anti-inflammatory, Anesthetic, Anticataract, Wound healer, Antidiarrheal, Antidiabetic, Antibacterial, Antifungal, Antiallergic, Antileprosy, Antipyretic | 107 |
| | 7-Methyl-*Z*-tetradecen-1-ol acetate | 23.86 | 0.54 | $C_{17}H_{32}O_2$ | 268 | Antihistaminic, Antieczemic, Hypocholesterolemic, Anti cancer, Anti-inflammatory | 108 |
| 28. | Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[*O*-(phenylmethyl) oxime], (3à,5à) | 26.24 | 0.99 | $C_{29}H_{43}NO_3Si$ | 481 | Antiasthmatic, Anti-inflammatory, Anticancer, Diuretic, Antimicrobial | 109 |

***PA** (Peak Area), **RT** (Retention Time), **MW** (Molecular weight) and **MF** (Molecular Formula)*

**Table III: FTIR peak values and functional groups in leaves of *D. montana***

| S. No. | Frequency range ($cm^{-1}$) | Peak value ($cm^{-1}$) | Functional group | Compounds[113,114] |
|---|---|---|---|---|
| 1. | 3600-3200 | 3416.5 | O-H stretching | Aromatic hydroxyl |
| 2. | 3000-2850 | 2924.3 | C-H stretching | Aliphatic alkanes |
| 3. | 3000-2850 | 2855.0 | C-H stretching | Aliphatic alkanes |
| 4. | 1680-1620 | 1640.6 | C=C stretching | Cyclic alkene |
| 5. | 1400-1000 | 1381.7 | C-H bending | Aromatic alkane |
| 6. | 1320-1000 | 1319.8 | C-N stretching | Aromatic amine |
| 7. | 1320-1000 | 1050.3 | C-O stretching | Alcohols, carboxylic acids |
| 8. | 910-665 | 778.9 | C-H bending | 1,3-disubstituted (Meta) |
| 9. | 700-600 | 631.6 | C-Br stretching | Aliphatic bromo compound |
| 10. | 600-500 | 520.2 | C-I stretching | Aliphatic iodo compound |

and heated gasses of individual substances were carried out to column (an inert gas). The mass spectrum was taken at 70 eV in 50-700 range along with MS transfer time temperature of 250 °C and ion source temperature was 230 °C.

### Identification of compounds

In gas chromatography, heat separates the compound mixture into different components and each compound was interpreted using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in NIST library. Bioactivity of different compounds was recorded from the available literature[28].

### Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy is based on the vibrational frequencies which are specific for each functional group. It corresponds to specific chemical bond when fall under specific region and organic molecule can be identified by detecting the characteristic frequency absorption band in the infrared spectrum[29]. Different functional groups have been observed in the leaf plant powder. FT-IR spectroscopy has been done using the Thermo Scientific, Nicolet iS50 FT-IR under 400-4000 $cm^{-1}$ infrared region according to ATR method at CIL/SAIF, Panjab University, Chandigarh.

## RESULTS AND DISCUSSION

### Phytochemical screening

The preliminary screening of phytochemicals in different parts (stem, leaf and seed) of *D. montana* using aqueous, ethanol, acetone and hexane extracts has been carried out. The analysis has shown the presence of a variety of phytoconstituents in different parts of the plant in a variable amount (Table I). Carbohydrates, cardiac glycosides, glycosides, proteins, quinones, starch, steroids and terpenoids were present in all plant extracts whereas amino acid was absent in these extracts. Similarly, anthocyanin was found in all extracts except stem and leaf extract of hexane. Alkaloids, anthraquinones, phenols and tannins were present only in aqueous, ethanol and acetone extracts. Gum and mucilage and saponins were found exclusively in all aqueous extracts. The ethanol extract of leaves has shown better results. It is likely because of high polarity of the solvent that has extracted maximum metabolites. Water is easily available, affordable and universal solvent but could not extract all available compounds within the plant materials. To overcome this, organic solvents like ethanol, acetone and hexane have been used. Organic solvents differ from water in solubilizing, thus they extract additional compounds[30].

Phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, tannins, phenolics, triterpenoids phytosterol and steroids are found in petroleum ether, chloroform and ethyl acetate extracts of *D. montana* leaves[31]. Phytochemicals such as alkaloid, flavonoids, phenols, carbohydrates, glycosides, proteins, saponins, steroids, terpenoids and tannins were reported in aqueous extract of *D. montana* leaves[15]. Findings of these previous studies have corroborated the present study on phytochemicals screening in the leaves, but carried out in different solvents.

Phytoconstituents such as betaxanthin, cardiac glycosides, coumarins, diterpenoids, gum and mucilage, oxalate, proteins, quinones, reducing sugar and resin have

not been reported in the earlier studies. Thus it is a unique addition to the phytochemical account of *D. montana*. Alkaloids are known to be anticonvulsant, hypotensive, antispasmodic, antimicrobial and antidiabetic[32,33]. Flavonoids possess antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiviral, antioxidant, anticancer and antitumor activities[34]. The saponins serve as an antibacterial, hemolytic and cholesterol binder[35]. Phenolic, tannins, steroids and terpenoids are associated with antimicrobial, antiviral, antidiabetic and cytotoxic activities[36]. Carbohydrates, glycosides and coumarins are immunity boosters, thus provide strength to the body. Most of these phytochemicals have been reported in extracts of different plant parts of *D. montana*. It shows that this plant or its parts can play a vital role as anticancer, antidiabetic, antioxidant, antimicrobial, anti-inflammatory agent are more.

## Gas chromatography-Mass spectroscopy

The ethanol extract of leaves of *D. montana* was used to carry out GC-MS analysis. The mass chromatogram has depicted 42 peaks, corresponding to similar number of chemical compounds. According to the available literature, out of 42 compounds, 28 compounds possess one or more bioactivities. The reported compounds have reflected the biological activities of plant from which these have been extracted (Fig. 2, Table II). Twenty three (23) compounds of the present study including (+)-2-bornanone, tridecane, cyclohexane, (4-methylpentyl)-, cyclooctasiloxane, hexadecamethyl-, oxirane, hexadecyl- etc. are antimicrobial in nature[37,38]. A strong antimicrobial activity of phytol against bacterial and fungal strains had also been reported[39]. Compounds like (+)-2-bornanone, camphor, tridecane, phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, eicosanoic acid, cis-vaccenic acid, trans-13-octadecenoic acid, 7-methyl-Z-tetradecen-1-ol acetate, androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[o-(phenylmethyl) oxime], (3à,5à), Trans-13-Octadecenoic acid have been reported as anticancerous thus inhibit the growth of proliferating cancer cells[38,40]. These compounds have also been reported in the present study. Similarly, 3,5-dithiahexanol 5,5-dioxide, (+)-2-bornanone and oleic acid compounds have antitumor properties. Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans, dodecane, 5-eicosene, (*E*)-, 5-anilino-1,2,3,-thiadiazol, oxirane, hexadecyl- etc. are antioxidants. The compounds present in *D. montana* have anticancer as well as antioxidant activities, thus reduce the free radicals and prevent cell damage. The compounds such as 9,12,15 octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1[(trimethylsilyl) oxy]methyl]ethyl ester, (*z, z, z*)-,1-monolinoleoylglycerol trimethylsilyl ether, oxirane, [(hexadecyloxy) methyl]- are potent antidiabetics[41]. 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-, undecane, phytol, oxirane, hexadecyl-, n-hexadecanoic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, eicosanoic acid, oxirane, [(hexadecyloxy) methyl]- etc. were found to be anti-inflammatory, antibacterial, antifungal and antiviral[42,43]. In addition to the above, leaves of *D. montana* possess antiasthmatic, antiandrogenic, antimetastatic, anticholesterol and other activities.

GC-MS analysis of *D. montana* (roots) revealed 100 bioactive compounds[44]. The difference in the number of reported compounds may be due to the use of roots and methanol as solvent. The roots are generally not preferred to be used because uprooting of plant species may pose threat to its survival and conservation. A variety of chemical compounds reported in the ethanol extract of leaves of *D. montana* during the present study have established the use of this species in folk medicines and further studies are recommended. However, to the best of our knowledge, this is the first study to identify the bioactive constituents of *D. montana* ethanol leaf extract by GC-MS analysis.

## FT-IR analysis

The Fourier transform infrared (FT-IR) spectroscopy has yielded different peaks corresponding to the functional groups (Fig. 3). The IR characteristic fingerprint peaks for *D. montana* are in the range of 3416.5 to 520.2 $cm^{-1}$. The infra-red spectrum shows a frequency range which corresponds to different stretching vibration and their functional groups such as 3416. 5 $cm^{-1}$ (O-H); 2924.3-2855.0 $cm^{-1}$ (C-H); 1640.6 $cm^{-1}$ (C=C); 1381.7 $cm^{-1}$ (C-H); 1319.8 $cm^{-1}$ (C-N); 1050.3 $cm^{-1}$ (C-O); 778.9 $cm^{-1}$ (C-H); 631.6 $cm^{-1}$ (C-Br); 520.2 $cm^{-1}$ (C-I) (Table III). Different functional groups were described through FT-IR in the leaves of *D. montana* including O-H stretching, C-H stretching, C=C stretching, C-H bending and C-O stretching at slightly different peak values[15]. These functional groups coincide with the reported compounds in *D. montana.* The O-H group is responsible for the antibacterial activity. Phenolic compounds contain benzene ring with one or more hydroxyl groups as stress reliever, antimicrobial agent and protect from pathogenic attack[110]. Similarly, amines are an integral part of amino acids and subsequently protein of living beings[111]. Carboxylic acid functional group in plant sample is anti-inflammatory and antimicrobial[112]. This study has endorsed the findings of the present study. The presence of alcohol, alkanes, alkenes, amines, carboxylic acid, ether, phenol etc. has substantiated the medicinal importance of *D. montana* leaves.

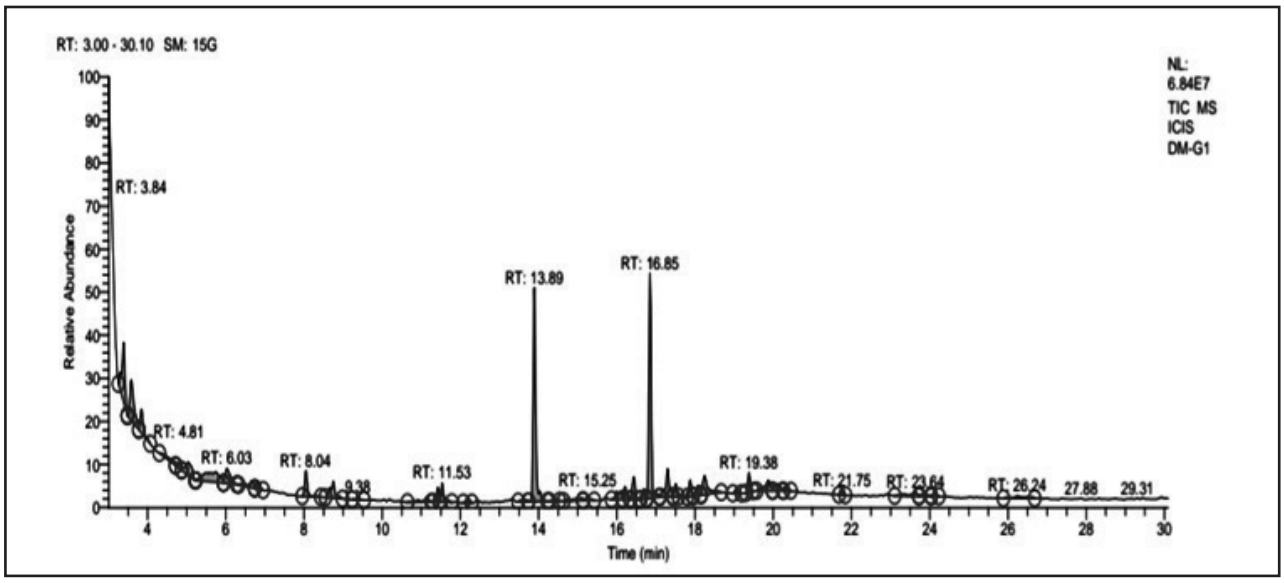


**Fig. 2: GC-MS chromatogram of ethanol extract of leaves of *D. montana***

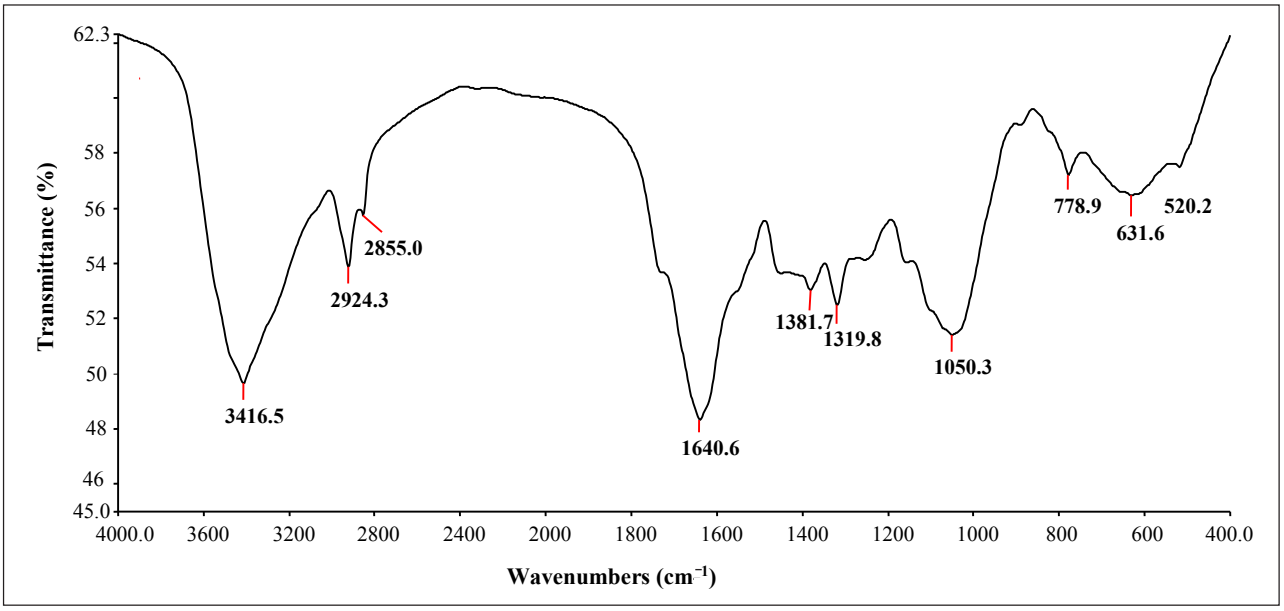


**Fig. 3: FTIR spectrum of *D. montana* leaves**

## CONCLUSION

*D. montana* has been depicted in the literature as an important traditional medicinal plant. The presence of phytochemicals like alkaloids, anthocyanins, carbohydrates, flavonoids, tannins, terpenoids, steroids etc. results in bioactivity of *D. montana* against various human health troubles. The major phytoconstituents present in plant or its specific parts can be utilized as per the requirement while considering the health of plant and its conservation. The GC-MS analysis has revealed the presence of 42 compounds in ethanol extract of leaves. Tridecanoic acid, camphor, phytol, eicosanoic acid, oleic acid, trans-13-octadecenoic acid etc. are anticancerous compounds. Similarly, the compounds like cyclohexane, (4-methylpentyl), cyclohexane, (4-methylpentyl)-, phytol, 1-monolinoleoylglycerol trimethylsilyl ether, 9,12,15-octadecatrienoicacid,2-[(trimethylsilyl)oxy]-1[[(trimethylsilyl)oxy]methyl]ethyl ester,(*z,z,z*)- and oxirane, [(hexadecyloxy)methyl]- are antidiabetic. Rest of the compounds possess activities including anti-inflammatory, antimicrobial, antioxidant etc. The FTIR analysis has provided a detail of the functional groups related to alcohols, aliphatic compounds, alkanes,

alkenes, amine, carboxylic acid etc. The phytochemical characterization has advocated the medicinal prospective of the *D. montana*. Further studies related to isolation, identification and bioactivity of these compounds are required.

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