*Asian J. Adv. Basic Sci.:* **2017, 5***(2),* 29-33
***ISSN (Print): 2454 – 7492***
***ISSN (Online): 2347 – 4114***
***www.ajabs.org***

# Phytochemical Investigation of *Dicoma tomentosa* to Isolate Secondary Metabolites

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*(Received 29 Aug, 2017; Accepted 14 Sept, 2017; Published 21 Sept, 2017)*

**ABSTRACT:** ***Dicoma tomentosa*** **Cass. (Family: Asteraceae) is an erect, annual herb of dry fields and sandy arid places. The whole plant is strongly bitter and is used for different ailments. Phytochemical investigation of the aerial part of plant was done to screen its secondary metabolites. Sesquiterpene lactones belonging to melampolide group of germacranolides were found to be characteristic of the species and were isolated along with triterpenes and steroids.**

**Keywords:** *Dicoma tomentosa;* Natural products; Sesquiterpenoids; Spectral analysis.

**INTRODUCTION:** Several products isolated from plants, marine flora and microorganisms possess the unique properties like greater number of chiral centers, increased steric complexity, fewer hetero atoms, fewer heavy atoms etc. It accounts for the huge structural diversity of natural products which further results into its rich bioactive potentiality[1]. The genera *Dicoma* belonging to the family Compositae, tribe- Mutisieae and subtribe- Gochanatiinae[2] is of great medicinal and economic importance. For intangible, physiological and structural reasons, the group seems to be well adapted to many habitats such as deserts, which are difficult for other plants. As a result the Compositae includes innumerable successful species[3].A large numbers of plants are ornamental and cultivated in gardens and homes. Some of them have medicinal importance. The genus *Dicoma* with approximately 35 species distributed over tropical South Africa with one species in Asia. Most of species are herbs or small shrubs but even trees are known. *D. tomentosa* Cass. is used medicinally in Western Africa; *D. anomala* Sond., *D. capensis* Less., *D. speciosa* E. May., *D. zeyheri* Sond are used in South Africa[4]. *D. tomentosa* is used as tooth cleaner[5]. Its leaves are used in the neighbourhood of Belgium as a febrifuge, during febrile attacks in the mother after child birth[6]. In Africa it is used as a local application to putrescent wounds. It is reported to possess prominent antiplasmodial activity[7-9]. The versatile features of secondary metabolites prompted us to study these phytochemicals from the plant *D. tomentosa.* The shed air dried aerial part of the plant was screened chemically to investigate its secondary metabolites.

**MATERIALS AND METHODS:**

**General experimental procedures:** Active neutral (Acme Synthetic Chemicals) and silica gel (BDH, 60-120 mesh) were used as adsorbent for column chromatography. Qualitative and quantitative thin layer chromatographies were conducted on TLC aluminium sheets Kieselgel 60 $F_{254}$ (E. Merk). Preparative TLC performed on TLC glass plates silica gel 60 $F_{254}$ precoated (20×20 cm) layer thickness 0.5mm (E. Merk). Spots on TLC plates were visualized by spraying with 2% cerric ammonium sulphate in 2N $H_2SO_4$.

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected. The identities of compounds were confirmed by comparison of their melting points and spectral data with literature values and also by mixed mp, co-TLC and co-IR with authentic samples.

The reported UV and IR spectra were recorded on Hitachi U-2000 double beam spectrophotometer and FT-IR spectrophototometer Magna IR-550 respectively. $^1H$ and $^{13}C$ NMR spectra were recorded on JEOL FX 90 Q, JEOL AL 300, Bruker DRX 300 and Bruker WM 400 instruments using $CDCl_3$, $C_6D_6$, $d_6$ DMSO as solvents and TMS as an internal reference. EIMS, CIMS and FABMS data were generated on JEOL D-300, Shimadzu QP-5000 and JEOL-JMS-SX 102A spectrometers respectively.

Plant materials were collected from Sawai Madhopur and Jaipur regions of Rajasthan and it was characterized by the Herbarium In-charge, Department of Botany, University of Rajasthan, Jaipur.

**Extraction and isolation of constituents from aerial parts of *Dicoma tomentosa*:** Air dried and coarsely powdered plant material (3kg) was extracted with $CH_2Cl_2$-ether-mathanol (1:1:1) mixture in cold for 12 hrs. The extract was concentrated at reduced pressure

and subjected to column chromatography over silica gel. The eluants used and fractions obtained are cited in table 1 to isolate different secondary metabolites from the plant material (Figure 1).

**Table 1: Eluants and the fractions from column chromatography.**

| Fraction No. | Eluant | Residue left after removal of solvent | |
|---|---|---|---|
| | | Nature | Amount |
| 1. | Petroleum-ether | Yellow Oil | 150 mg |
| 2. | Petroleum-ether: Benzene (3:1) | Yellow semisolid | 160 mg |
| 3. | Petroleum-ether: Benzene (1:1) | Yellow solid | 500 mg |
| 4. | Benzene | Yellow green solid | 150 mg |
| 5. | Benzene: EtOAc (3:1) | Yellow gum | 200 mg |
| 6. | Benzene: EtOAc (1:1) | Yellow gum | 190 mg |
| 7. | EtOAc | Yellow solid | 120 mg |

**RESULTS AND DISCUSSION:** The air–dried and coarsely powdered aerial parts were extracted with dichloromethane–ether–methanol (1:1:1) mixture in cold. The resulting extract was concentrated at reduced pressure and chromatographed over silica gel. Subsequent fractions (Table 1) on preparative TLC gave following compounds.

Fraction 1 could not be investigated further on account of complex mixture of several non polar compounds as evidenced by the appearance of trailing on TLC plate. Fractions 2-7 were found to be a mixture of two or more compounds and each fraction was subjected to preparative TLC to get compounds in pure state.

**Component from fraction 2:** Fraction 2 showed a major spot along with some very minor spots on qualitative TLC plate. It was therefore purified by preparative TLC using petroleum-ether: benzene (2:1) as solvent system.

**1. Isolation of Lupeol acetate:** It was isolated as colourless crystals 110 mg, mp 218-19 $^0$C and belongs to lupane series of triterpenes. It responded to positive Noller test for triterpenes.

| | |
|---|---|
| IR $\nu_{max.}$ [KBr] $cm^{-1}$ | 1725 (OAc) , 1640 (C=C) , 1160 , 990 $cm^{-1}$ (C-O stretch). |
| $^1$H NMR [300 MHz, $CDCl_3$, δ ppm] | 1.69s (Me-C=C), 4.69 sbr and 4.57 s (Vinylic protons), 4.38dd (J=12, 5HZ, H-3), 2.38m (H-19), 2.10s (OAc), 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 (all singlets representing six methyl groups) . |
| MS (m/z) | 468 $[M]^+$ ($C_{32}H_{52}O_2$ ) , 453 [ M-Me ]$^+$ , 450 [ M-$H_2O$]$^+$ , 408 [ M-AcOH ]$^+$ , 242, 240, 231 and 213, etc. |

**Components from fraction 3:** Fraction 3 revealed the presence of two compounds on TLC plate. These compounds were separated by prep. TLC in petroleum-ether: benzene (1:1) mixture.

**2. Isolation of Stigmasterol:** It was the second component separated from fraction 3 and was obtained as shining needles, 175mg. mp 166-67 $^0$C after crystallization from $CHCl_3$ – methanol (1:1) mixture. It gave positive Liebermann–Burchard and Salkowski test for sterol and TNM test for unsaturation.

| | |
|---|---|
| IR $\nu_{max.}$ KBr | 3400-3200 (OH), 1460 (-CH=CH- bending), 1380, 1360, 1260, 1050, 960, 800 $cm^{-1}$. |
| $^1$H NMR [300 MHz | 5.34 tbr (H-6), 5.04 dd(J=16, 10 Hz, H-22), 5.12 dd (J=16,10 Hz, H-23), 3.52 m (H-3α), 0.81 t (J=7 Hz, C-29 methyl), 0.91 d (J=7 Hz, C-21 methyl), 1.56 s (OH), 1.16 s (C-27 methyl), 0.88 s (C-19 methyl), 0.79 s (C-18 methyl). |
| MS (m/z) | 412 $[M]^+$ ($C_{29}H_{48}O$), 397 [M-Me]$^+$, 328, 314, 302, 300, 271, 253. |

**3. Isolation of β-Sitosterol**: Yellowish solid obtained from fraction-3 after removal of solvent gave a colourless solid, 275 mg which was crystallized from benzene as colourless flaps, mp 136-37 $^0$C and characterized as β–sitosterol.

**Components from fraction 4:**

**4. Isolation of Betulin:** The fraction 4 afforded yellowish solid after removal of solvent. After crystallization from $CHCl_3$–methanol (1:1) betulin was isolated as colourless needles, 120 mg, mp 253-54 $^0$C (lit 254-56 $^0$C). It belongs to lupane series of triterpenes and gave positive Noller test. It also developed yellow colour with tetranitromethane indicating its unsaturated nature.

**Components from fraction 5:** The fraction 5 showed the presence of three compounds on TLC plate. Recolumn chromatography followed by prep. TLC of this fraction afforded following compounds

**5. Isolation of Taraxasterol:** It was obtained from first fraction of re-column chromatography and was crystallized from methanol as white needles, 40 mg, mp 225-226 $^0$C**.** It was highly soluble in benzene, chloroform and acetone. It responded positively to TNM test (yellow colour). It also gave pink-violet colour with Libermann-Burchard reagent and red colour with chlorosulphonic acid. It also responded positively to Salkowski and Noller tests. It displayed following spectral details:

| | |
|---|---|
| IR $\nu_{max}$ [KBr] | 3470 (OH), 2980, 2960, 2855 (C-Hstretch), 1650 (c=c stretch), 1460, 1375 (gem dimethyl groups) and 1050 $cm^{-1}$ (c-o stretch). |
| $^1$H NMR [300 MHz, $CDCl_3$, δ(ppm)] | 0.88 s, 0.87 s, 0.86 s, 0.85 s, 0.84 s, 0.83 s, 0.82 d (J=7 Hz) (these signals corresponded to seven methyl groups), 4.56 m (exomethylene protons), 3.23 dd (J=7,11 Hz, H-3). |
| MS (m/z) | 426$[M]^+$ ($C_{30}$ $H_{50}$ O), 411 $[M\text{-}Me]^+$, 408$[M\text{-}H_2O]^+$, 203 etc. |

**6. Isolation of 8-Desoxyurospermal A -15-O-acetate**: It was the second component isolated from fraction 5. It was obtained as colourless gum, 70 mg. It gave single spot on TLC plate. Its melting point could not be determined due to its sticky nature. It displayed following spectral details:

| | |
|---|---|
| IR $\nu_{max}$ $CHCl_3$ $cm^{-1}$ | 2950-2840, 1775 (γ-lactone), 1740 (OAc), 1670, 1635 (C=C-CHO), 1470, 1390, 1320, 1240, 1145, 1100, 1040, 980, 910. |
| $^1$H NMR [300 MHz, $CDCl_3$, δ(ppm)] | 6.52 ddd (J=7, 8.5, 1.5 Hz, H-1), 2.49 m (H-2), 2.32 m (H-2'), 2.61 ddd (J=6, 2, 12.5 Hz, H-3), 2.11 brdd (J=12, 12.5Hz , H-3'), 5.29 brd (J=10Hz, H-5), 4.67 dd (J=10, 11Hz, H-6), 2.28 ddddd (J=3, 3.5, 7, 10, 11Hz, H-7), 2.95 dddd (J=3, 10, 6.5, 12Hz, H-8), 1.52 m (H-8'), 2.43 brdd (J=10, 14Hz, H-9), 2.5 m (H-9'), 6.20d (J=1.5Hz, H-13), 5.50 d (J=1.5Hz, H-13'), 9.48 s (H-14), 4.79 d (J=12.5 Hz, H-15), 4.71 d (J=12.5 Hz, H-15'). |
| MS m/z (rel. int %) | 304 $[M]^+$ (0.1) ($C_{17}H_{20}O_5$), 244.110 $[M\text{-}HOAc]^+$ (100), 216 $[244\text{-}CO]^+$ (24), 215 $[244\text{-}CHO]^+$ (37). |

**7. Isolation of 8-Desoxyurospermal A:** It was the third component isolated from fraction 5 after recolumn chromatography. It was obtained as colourless gum, 80 mg. It belongs to melampolide group of sesquiterpene lactones. It exhibited following spectral data:

| | |
|---|---|
| IR $\nu_{max}CHCl_3$ $cm^{-1}$ | 3500 (OH), 2970-2850, 1770 (γ-lactone), 1690-1630 (C=CCHO), 1600, 1380, 1285, 1175, 1100, 1070, 940, 900. |
| $^1$H NMR [300MHz , | 6.54 ddd (J=7, 9, 1.5 Hz, H-1), 2.49 m (H-2), 2.33 m (H-2'), 2.76 ddd (J=6, 2, 12 Hz, H-3), 2.03 ddd (J=3,11,12 Hz, H-3'), 5.18 brd (J=10 Hz, H-5), 4.76 dd (J=10, 11 Hz, H-6) , 2.28 ddddd (J=3, 3.5, 6.5, 10, 11 Hz, H-7), 2.94 dddd (J=3, 10, 6.5, 12 Hz, H-8), 1.52 dddd (J=3.5, 10, 6.5, 12 Hz, H-8'), 2.42 ddd (J=6.5, 10, 14 Hz, H-9), 2.11 ddd (J=6.5, 10, 14Hz, H-9'), 6.20 d (J=1.5 Hz, H-13), 5.50 d (J=1.5Hz,H-13'), 9.48 s (H-14), 4.47 d (J=12.5 Hz, H-15), 4.29 d (J=12.5 Hz, H-15'). |
| MS m/z (rel.int.%) | 262 $[M]^+$ (2) ($C_{15}H_{18}O_4$), 224.110 $[M\text{-}H_2O]^+$ (8) ($C_{15}H_{16}O_3$), 216 $[244\text{-}CO]^+$ (12), 215 $[244\text{-}CHO]^+$ (15), 53 (100). |

**Components from fraction 6:** Fraction 6 of column chromatography on evaporation of solvent furnished a residue. It showed the presence of two components, close in their $R_f$ values. It was therefore, subjected to prep. TLC (benzene: $CH_2Cl_2$: ether, 4 : 4 : 1) to get compounds in pure state.

**8. Isolation of Urospermal A-15-O-acetate:** The first band of prep. TLC afforded urospermal A-15-O-acetate as colourless solid, 60 mg. It belongs to melampolide group of sesquiterpene lactones. Its spectral analysis showed the following results.

| | |
|---|---|
| IR $\nu_{max}CHCl_3$ $cm^{-1}$ | 3420 (OH), 2950-2840, 1770 (γ-lactone), 1750 (OAc), 1690, 1630 (C=CCHO), 1420, 1240, 1170 (C-H bending), 1140, 1030, 980, 900 (C=$CH_2$ bending). |
| $^1$H NMR [300 MHz, $CDCl_3$, δ(ppm)] | 6.78 brdd (J=7, 9 Hz, H-1), 2.55ddd (J=6, 2, 12 Hz, H-3), 2.25 brdd (J=11,12 Hz, H-3'), 5.15 br d (J= 10 Hz, H-5), 4.51 dd (J=10, 11 |

| | |
|---|---|
| | Hz, H-6), 2.49 dddd (J=3, 3.5, 10, 11 Hz, H-7), 3.91 ddd (J=3, 10, 6.5 Hz, H-8), 2.51 m (H-9), 6.45 dd (J=1.5, 3.5 Hz, H-13), 6.23dd (J=1.5, 3 Hz, H-13'), 9.39 s (H-14), 4.74 d (J=12.5 Hz, H-15), 4.63 d (J=12.5, H-15'), 5.64 d (J=11 Hz, OH), 2.10 s (three protons of acetate group). |
| MS m/z (rel. int. %) | 320 $[M]^+$ (0.5) ($C_{17}H_{20}O_6$), 260 $[M\text{-}HOAc]^+$ (55), 242 $[260\text{-}H_2O]^+$ (25), 214 $[242\text{-}CO]^+$ (31), 213 $[242\text{-}CHO]^+$ (31), 69 (100). |

**9. Isolation of Urospermal A:** The second band of prep. TLC gave urospermal A as colourless crystalline solid, 80 mg, mp 163-165 $^0$C. Spectroscopic studies showed the following result:

| | |
|---|---|
| IR $\nu_{max}$ $CHCl_3$ $cm^{-1}$ | 3540 (OH), 2990-2850, 1770 (γ-lactone), 1680,1635 (C=C-CHO), 1645, 1440, 1280, 1150, 1080, 960, 900 $cm^{-1}$. |
| $^1$H NMR[300 MHz, $CDCl_3$, δ(ppm)] | 6.86 brdd (J=7, 9 Hz, H-1), 2.63 m (H-2), 2.37 m (H-2'), 2.65ddd (J=6, 2, 12 Hz, H-3), 2.8 brdd (J=11, 12 Hz, H-3'), 5.09 br d (J= 10 Hz, H-5), 4.57 dd (J=10, 11 Hz, H-6), 2.45 dddd (J=3, 3.5, 10, 11 Hz, H-7), 3.87 ddd (J=3, 10, 6.5 Hz, H-8), 2.49 m (H-9), 6.52 dd (J=1.5, 3.5 Hz, H-13), 6.33 dd (J=1.5, 3 Hz, H-13'), 9.45 s (H-14). |
| MS (m/z) | 278 $[M]^+$ ($C_{15}H_{18}O_5$), 260 $[M\text{-}H_2O]$+, 232 $[260\text{-}CO]^+$, 231 $[260\text{-}CHO]^+$. |

**Components from fraction 7:**

**10. Isolation of Betulinic acid:** Fraction 7 revealed the presence of single major compound on TLC examination. It was obtained as colourless crystals, 90 mg, mp 314-316 $^0$C. It answered positive Noller's test. It furnished following spectral data:

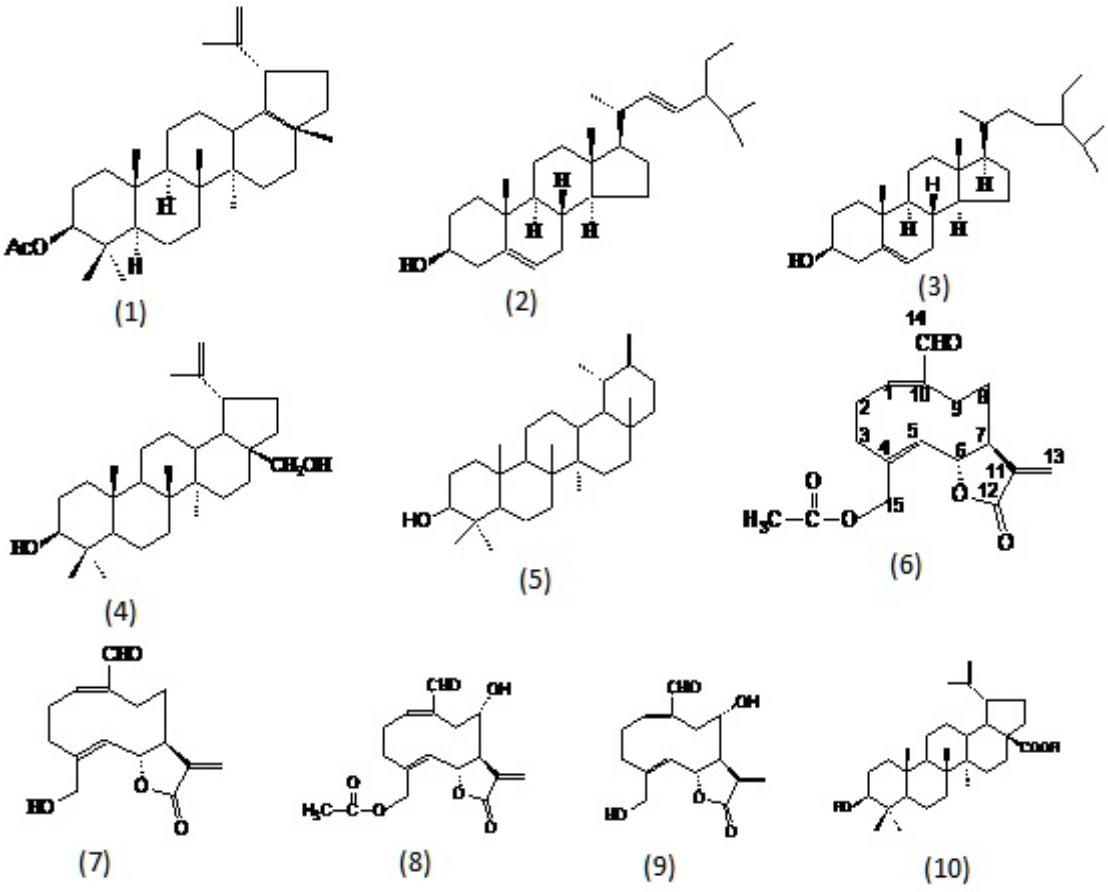


**Figure 1: Isolated secondary metabolites from the aerial part of *D. tomentosa.***

| | |
|---|---|
| IR $\nu_{max}$ KBr | 3425-2800 (OH, COOH), 1715(C=O),1640,900 $cm^{-1}$. |
| $^1$H NMR [90 MHz, $CDCl_3$, δ(ppm)] | 4.68 sbr and 4.56 sbr (=$CH_2$), 1.68 s (=C-$CH_3$), 2.30 m (H-19), 3.27 dd (J=12, 5 Hz, H-3α), 0.76 s (3H), 0.78 s (3H), 0.82 s (3H), 0.96 s (3H), 1.03 s (3H) for five tertiary methyl groups. |
| MS (m/z, rel. int. %) : | 456 $[M]^+$ ($C_{30}H_{48}O_3$), 438 $[M-H_2O]^+$ (22), 423 $[438-Me]^+$ (14), 411 $[M-COOH]^+$ (12), 410 (9), 342 $[M-C_6H_{10}O_2]^+$ (7.2), 248 (65), 220 (42), 207 (79), 203 (49), 189 (100), 149, 69, etc. |

**CONCLUSION:** The aerial part of *Dicoma tomentosa* was phytochemically screened to observe different natural products of the plant. Four highly oxygenated germacranolides, three triterpenes (among them two of lupane series), two steroids were isolated and characterized with the help of spectral studies.

**ACKNOWLEDGEMENT:** Author is thank full to Prof. Pahup Singh for his consistence guidance and Department of Chemistry, University of Rajasthan, Jaipur for providing all necessary facilities for the research work.

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