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Biological Importance of Phytoconstitents Isolated from the Genus Randia & GC-MS Analysis of Petroleum-Ether Fruit Extract of *Randia dumetorum*

MANISHA* and M. C. SHARMA

Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan 302004, India.
*Corresponding E-mail: manialaria1989@gmail.com

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ABSTRACT

Randia genus (Indigo berry) belongs to family Rubiaceae, native to America, allocated in tropical regions. Most of the Species associated with this Genus used as ornamental, folk medicine to treat the disease of renal, malaria, cancer, dysentery, snake-bite etc. All plant parts are used by tribes for various ailment treatments. The phytochemicals generally associated with this genus are terpenoids, saponins, lignans, coumarin, iso-coumarin, flavonoids, tannins, essential oil and acid resin. This review highlights the phytochemicals and pharmacological activity reports. Phytochemical investigation of *Randia dumetorum* fruit extract using petroleum ether solvent, reveals the presence of 2,4-di-tert-butylphenol, octadecadienoic acid, 23(Phenylsulfanyl) lanosta-8,24-dien-3-ol, hexadecenoic acid, gamma-sitosterol, 4-tert-butylcalix[4]arene, tetracontane, tetratetracontane and octacosanol etc. Compound 1-Octacosanol (A), 9-Octadecenoic acid (B) and gamma-Sitosterol (C) were isolated with the help of column chromatographic techniques and characterized by spectral studies i.e. ^{1}H NMR, ^{13}C NMR, IR and mass spectroscopy.

Keywords: *Randia*, Rubiaceae, Folk medicine, Phytochemistry, Pharmacology, GC-MS.

INTRODUCTION

Randia genus commonly known as Indigo berry, belongs to Rubiaceae family, sub-family Ixoroideae, tribe-Gardeniae, native to the america, mainly distributed in tropical and subtropical regions. This is a large and well-defined family, with more than 600 genera and about 13,500 species found worldwide[1-3]. This genus associated with more than 100 species. Out of them more than 10 species are ethnopharmacologically important. *Randia* echinocarpa, *Randia* matude, Randia aculeata, *Randia* hebecarpa, *Randia* ferox, *Randia* momantha are the most studied species[4]. Excessive ethnobotanical and ethnomedicinal study of root, bark, leaves, fruit and seeds of this genus are reported in literature.

Randia dumetorum (Cautonaregam spinosa) also known as 'Mainphal' (Hindi & Bengali) 'Vamanaphala'(Sanskrit), highly reputed medicinal plant. It is deciduous shrub with thorns or small tree (height up to 5m.), which is distributed up to 4000 ft altitude throughout the india[4]. Leaves are

oval, wrinkled, shiny & pubescent. Flowers are white, wrinkled solitary and possess honey like fragrance[5]. Randia fruit approx. 1.8-4.5 cm length, spherical, have persistent calyx-limb, yellowish brown coloured. Fruit contains large no. of seeds with the length of 0.4–0.6 cm, hard, compressed & brown[6].

Roots are used as insect repellent and insecticide. Bark is sedative, nervine and used to relieve the pain of bruises and febrile bone ache. Fruits have antiasthmatic, antitumor, analgesic, antispasmodic, antiallergic, antifertility, abortifacient & nauseant and also used as fish poison and detergent[5,7-8]. The dry fruit powder with fresh milk treats gastric troubles. During emesis 'Mainphal' doesn't show any side effect so it is considered as best emetic drug (vamaka dravya)[5]. Seeds show the antidiarrheal, antidysenteric activity. Seeds used to induce appetite and contain 14.2% protein, 1.5% fat, 1.4% organic acid, resin, mucilage[6,8].

Genus *Randia* exhibits a wide array of biological activities like anticancer, antioxidant, anti mutagenic, anti pyretic, anti venom, anti-inflammatory, hepatoprotective, abortifacient, haemolytic, molluscicidal, anthelmintic activities[4,8-16]. The phytoconstituents associated with this genus are flavonoids, lignans, sterols, coumarins, tannis, triterpenoids, saponins mainly.

Only *Randia* aculeata shows the antivenom effect[4]. Pseudogenosides and tyramine isolated from Randia siamensis are responsible for hypotensive and hypertensive activity respectively. Fruits of *Randia* echinocarpa (native to the maxico) consumed by rural areas as food/medicine[4].

Seeds of *Randia* monantha are fiber rich. Seed oil contain palmitic acid (21.78%), oleic acid (26.12%) & linoleic acid (46.56%)[17].

MATERIALS AND METHODS

Collection & pre-treatment of fruit sample

The fruit samples of *Randia dumetorum* fruit were collected from local market of Jaipur city Rajasthan (India). The fruit sample were washed, cleaned and dried. The plant's identification was confirmed by Dr. Mahesh C. Sharma (Retd. Professor), Department of Chemistry, University of Rajasthan, Jaipur.

Preparation of extracts and Column Chromatography

The air dried *Randia dumetorum* fruits were ground to fine powder using a grinder. Then the powdered fruits (approximately 1 kg) of *Randia dumetorum* were subjected to Soxhlet extraction procedure using Petroleum ether solvent (3ltr), for 12×3 days. "Rotary Vacuum Evaporator (N. N. Series) with a digital water bath SB-651 and an aspirator; Eyela, tokyo, Japan" was used to remove excess solvent and further sodium sulphate remove moisture from extract. Above extract was stored in air tight container at low temperature. It was filtered by Whatman No.1 filter paper to remove grainy matter and concentrated sample of extract was obtained. The extract was dissolved in petroleum ether (20-30 mg/mL) for GC-MS analysis. Fat free extract (after acetonitrile treatment) was subjected to column chromatographic separation over a silica gel column. Column elution was done by different solvent system according to increasing polarity.

GC-MS analysis

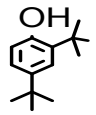
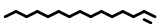
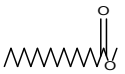
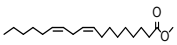
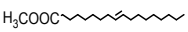
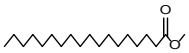
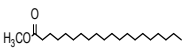
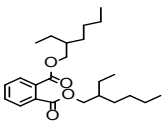
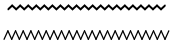
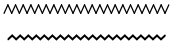
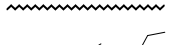
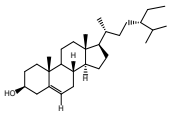
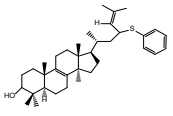
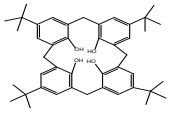
GC-MS analysis of *Randia dumetorum* fruit extract (Petroleum ether) was done at the Advanced Instrumentation Research Facility (AIRF) Lab, JNU, New Delhi using standard GCMS model as explained below.

Instrument details

Shimadzu GCMS-QP2010 Plus with thermal desorption system TD 20, AOC-20S auto-sampler & 20i auto-injector, was used to performed Gas chromatography-mass spectroscopy (mass range 1.5-1090 Daltons). The total run time of GC-MS was 29 minutes. Different compounds were detected by mass spectrometer according to their different retention time. A plot of intensity v/s retention time was recorded (Chromatogram). The compounds are identified by comparing the data with the WILEY8, NIST14 & NIST14s libraries with name, molecular formula, molecular weight and structures.

The GC spectrum of the petroleum ether extract shows total nineteen compounds, were determined by the chromatographic method with the help of NIST and WILEY library as shown in Table 2. Compound octadecadienoic acid was found to be in the highest concentration (13.36%) followed by hexadecenoic acid(11.63%), Octadecanoic acid(8.46%), 4tertbutylcalix[4]arene(5.16%), tetracontane (4.17%), tetratetracontane (3.40%), octacosanol (3.79%), other compounds were found in trace amount.

Table 1: Phytochemicals Identified in the Petroleum ether fruit extract of *Randia dumeorum* using GC-MS

Peak	Retention time	Mol.weight	Mol.formula	Area %	Compound name	Structure
1	8.980	206	$C_{14}H_{22}O$	1.20	2,4-Di-tert-butylphenol	
2	9.877	210	$C_{15}H_{30}$	1.84	1-Pentadecene	
3	9.957	226	$C_{16}H_{34}$	1.26	Hexadecane	
4	12.150	238	$C_{17}H_{34}$	2.75	1-Heptadecene	
5	12.215	254	$C_{18}H_{38}$	1.53	Octadecane	
6	13.555	270	$C_{17}H_{32}O_2$	11.63	Hexadecanoic acid,methyl ester	
7	14.204	266	$C_{19}H_{38}$	3.13	1-Nonadecene	
8	14.256	296	$C_{21}H_{44}$	1.63	Heneicosane	
9	15.202	294	$C_{18}H_{34}O_2$	13.36	9,12-Octadecadienoic acid (Z, Z), methyl ester	
10	15.256	296	$C_{19}H_{36}O_2$	8.46	9-Octadecenoic acid, methyl ester, (E)-	
11	15.476	298	$C_{19}H_{38}O_2$	2.99	Methyl stearate	
12	16.077	410	$C_{28}H_{58}O$	3.79	Octacosanol	
13	17.249	326	$C_{21}H_{42}O_2$	1.21	Eicosanoic acid, methyl ester	
14	17.795	410	$C_{28}H_{58}O$	3.66	Octacosanol	
15	18.943	390	$C_{24}H_{38}O_4$	1.35	1,2-benzenedicarboxylic acid	
16	19.381	410	$C_{28}H_{58}O$	3.10	Octacosanol	
17	20.156	618	$C_{44}H_{90}$	2.13	Tetratetracontane	
18	20.877	618	$C_{44}H_{90}$	3.40	Tetratetracontane	
19	21.579	562	$C_{40}H_{82}$	4.17	Tetracontane	
20	22.254	618	$C_{44}H_{90}$	3.11	Tetratetracontane	
21	22.994	562	$C_{40}H_{82}$	2.93	Tetracontane	
22	25.362	414	$C_{29}H_{50}O$	1.50	Gamma-sitosterol	
23	25.934	534	$C_{36}H_{54}OS$	2.00	23(Phenylsulfanyl)lanosta-8,24-dien-3-ol	
24	28.581	648	$C_{44}H_{56}O_4$	5.16	4-tert-butyl-calix[4]arene	

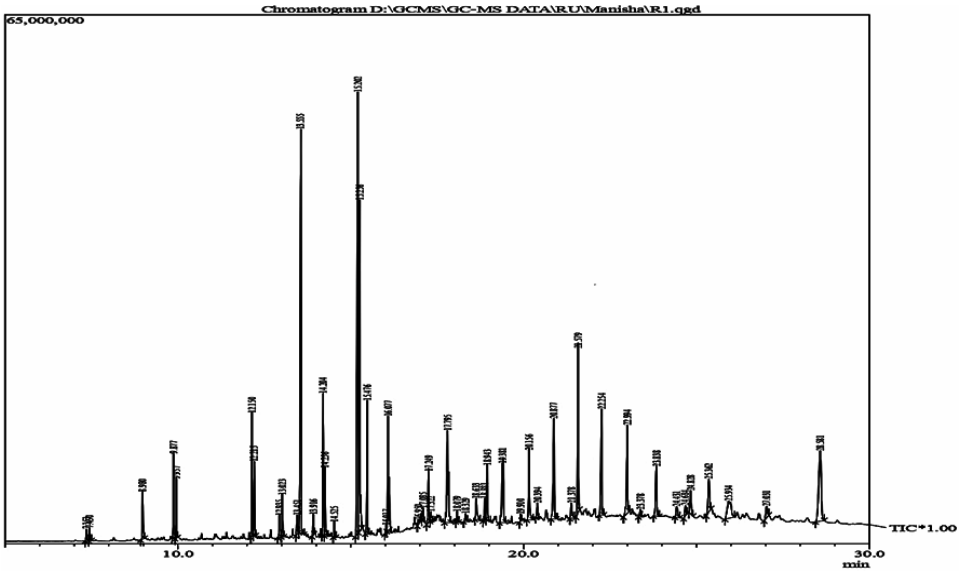


Fig. 1. GC-MS Spectrum of Pet ether fruit extract of *Randia dumetorum*

Isolation of compound A as 1-Octacosanol

Compound A was obtained by using hexane and dichloromethane with 1:4 as eluting solvents in column. After the solvents were eliminated the resultant material was crystallised with methanol to form colourless needle, m.p. 83-86°C. IR (KBr, cm^{-1}) 3300, 2899-2840, 1460, 1064, 734, 724. ^1H NMR ($CDCl_3$, δppm) 0.88 (3H, triplet), 1.25 (-CH_2, broad singlet), 3.57 (2H, triplet, -OCH_2). ^{13}C NMR ($CDCl_3$, δppm) 14.12(-CH_3), 22.69, 25.78, 29.36, 29.45, 29.61, 29.69, 31.92, 32.86 (each -CH_2), 63.14 (-CH_2OH). MS (m/z): 410 (M^+), 390, 364, 350, 308, 292 etc. Molecular formula calculated as $C_{28}H_{58}O$.

Isolation of compound B as 9-Octadecenoic acid

Cream-coloured oil obtained when column was eluted with pet-ether and benzene in ratio 3:1. m. p. 10-12°C. IR (KBr, cm^{-1}) 2950, 1712, 1620. ^1H NMR (δppm, $CDCl_3$) 10.48 (s, 1H), 2.23 (t, 2H, C-2), 1.58-1.62 (m, 2H, C-3), 1.28-1.30 (20H, s), 5.24 (2H, m), 0.93 (t, 3H). ^{13}C NMR spectrum (δppm, $CDCl_3$) 176.90 (C-1), 128.50 (C-9 and C-10), 32.3 (C-2, C-8, C-11), 25.10 (C-3), 28.30 (C-4), 28.65 (C-5, C-15), 29.15 (C-6, C-13, C-14), 29.75 (C-7, C-12), 30.66 (C-16), 21.82 (C-17), 13.89 (C-18). MS (m/z): 296 (M^+), 264, 222, 180 etc. Molecular formula calculated as $C_{19}H_{36}O_2$.

Isolation of compound C as γ-sitosterol

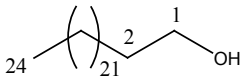
On eluting the column with chloroform white needle like crystals obtained. It showed m. p. 135-137°C, gave positive Liebermann burchard test. IR (KBr, cm^{-1}) 3445-3550, 1620, 1050. ^1H NMR ($CDCl_3$, δppm) 0.88 (3H, triplet), 1.25 (-CH_2, broad singlet), 3.57 (2H, triplet, -OCH_2). 5.32 (t, 1H, C-6), 3.50 (m, 1H, C-3), 0.66-2.42 (m, for remaining 26 protons). ^{13}C NMR spectrum (δppm, $CDCl_3$) 31.50 (C-1), 32.31(C-2), 42.23 (C-4), 32.51 (C-7), 46.33 (C-8), 49.73(C-9), 36.25 (C-10), 21.33 (C-11), 28.70 (C-12), 43.10 (C-13), 57.31 (C-14), 24.83 (C-15), 41.51 (C-16), 56.30 (C-17), 36.25 (C-20), 36.21 (C-22), 24.57 (C-23), 40.11 (C-24), 37.51 (C-25), 32.81 (C-28), 12.50 (C-18), 19.51 (C-19), 19.52 (C-21), 23.41 (C-26), 23.42(C-27) and 30.00 (C-29). MS (m/z): 414 (M^+), 397, 383, 369, 255 etc. Molecular formula calculated as $C_{29}H_{50}O$.

RESULTS & DISCUSSION

Isolation of compound A as 1-Octacosanol

Compound 1 was obtained by using hexane and dichloromethane with 1:4 as eluting solvents in column. After the solvents were eliminated the resultant material was crystallised with methanol to form colourless needle, m.p. 83-86°C. IR (KBr, cm^{-1}) 3300(-OH, stretching), 2899-2840 (C-H, stretching), 1460 (-CH_2-, bending), 1064 (C-O, stretching) and 734, 724 [-(CH_2) n-deformation, n>4]. ^1H NMR ($CDCl_3$, δppm) 0.88 (3H, triplet), 1.25 (-CH_2, broad singlet), 3.57 (2H, triplet, -OCH_2). ^{13}C NMR ($CDCl_3$, δppm) 14.12(-CH_3), 22.69, 25.78, 29.36, 29.45, 29.61, 29.69, 31.92, 32.86 (each -CH_2), 63.14

(-CH_2OH). MS (m/z): 410 (M^+), 390, 364, 350, 308, 292 etc. Molecular formula calculated as $C_{28}H_{58}O$.



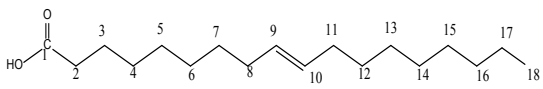
Characterization of compound B as 9-Octadecenoic acid

Cream-coloured oil obtained when column was eluted with pet-ether and benzene in ratio 3:1. m.p. 10-12^{0}C. Its IR spectrum (KBr, cm^{-1}) appeared at 2950 confirmed the presence of carboxylic group. The absorption at 1712 due to carbony group whereas the absorption at 1620 confirmed the presence of olefinic (C=C stretching) group.

The ^{1}H NMR spectrums (δppm, $CDCl_3$) showed a sharp singlet for one proton of carboxylic group at 10.48 (s, 1H, -COOH). A triplet at 2.23 (t, 2H, C-2) was assigned for C-2 protons and a multiplet was observed at 1.58-1.62 (m, 2H, C-3) due to protons of C-3 position. A broad singlet at 1.28-1.30 integrated for 20 protons of ten methylene group at C-4 to C-7 and C-12 to C-17 respectively. A multiplet observed at 1.89-1.95 was assigned to the protons of methylene present at C-8 and C-11 positions. Protons attached to olefinic carbon at position C-9 and C-10 showed a multiplet at 5.24. A triplet at 0.93 (t, 3H, C-18) for three protons of methyl group at C-18 position.

The ^{13}C NMR spectrum (δppm, $CDCl_3$) showed absorption at 176.90 was confirmed the presence of -COOH group at C-1 position. The olefinic group was confirmed by the absorption at 128.50 which were assigned to C-9 and C-10 carbon positions. Other peaks were obtained at 32.3 (C-2, C-8, C-11), 25.10 (C-3), 28.30 (C-4), 28.65 (C-5, C-15), 29.15 (C-6, C-13, C-14), 29.75 (C-7, C-12), 30.66 (C-16), 21.82 (C-17), 13.89 (C-18).

From the above spectral data, it was identified as compound "2" 9-Octadecenoic acid.



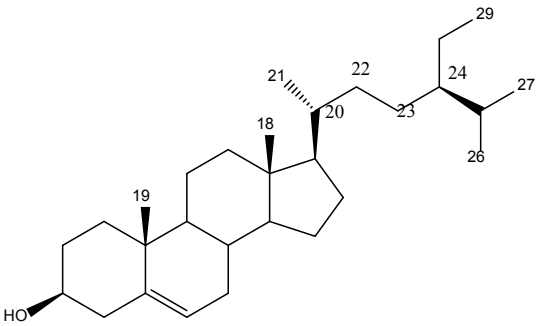
9-Octadecenoic acid Characterization of Compound C as *gamma*-Sitosterol

The compound 3 was isolated as white powder, gave positive Liebermann-Burchard and Salkowski tests characterstic for sterols. The mass spectrum exhibited the molecular ion peak at m/z 414 $[M]^+$ corresponding to its molecular composition as $C_{29}H_{50}O$. Other prominent peaks were observed at m/z 397, 383, 369, 255, etc.

In the IR spectrum (KBr, cm^{-1}), strong absorptions at w_{max}3445-3550 showed the presence of the hydroxyl group. Other absorptions at 1620 and 1050 were characterized for olefinic group (C=C stretching) and for C-O stretching respectively.

The ^{1}H NMR spectrum (δppm, $CDCl_3$), exhibited abroad triplet at 5.32 corresponding to H-6 olefinic proton. A multiplet appeared at 3.50 for H-3 α-proton. The rest of the protons of compound appeared in high field region 0.66-2.42.

In ^{13}C NMR spectrum (δppm, $CDCl_3$), absorption at 72.21 demonstrated the presence of a hydroxyl group at C-3 position and two absorptions at 141.31(C-5) and 123.52 (C-6) can be assigned to olifinic carbons respectively. The assignments of other carbon atoms and their position were established as 31.50 (C-1), 32.31(C-2), 42.23 (C-4), 32.51 (C-7), 46.33 (C-8), 49.73(C-9), 36.25 (C-10), 21.33 (C-11), 28.70 (C-12), 43.10 (C-13), 57.31 (C-14), 24.83 (C-15), 41.51 (C-16), 56.30 (C-17), 36.25 (C-20), 36.21 (C-22), 24.57 (C-23), 40.11 (C-24), 37.51 (C-25), 32.81 (C-28), 12.50 (C-18), 19.51 (C-19), 19.52 (C-21), 23.41 (C-26), 23.42 (C-27) and 30.00 (C-29).



γ-sitosterol

CONCLUSION

Defatted petroleum-ether extract produced 1-octacosanol (A), Octadecanoic acid (B) andv γ-sitosterol (C). In view of the immense biological, nutraceutical and pharmacological importance of the genus randia, we have systematically reviewed this genus as it may be helpful to food industry and

pharmaceutical as well as phytochemists, biologists and pharmacologists.

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Conflicts of interest

Present study does not contain any conflict of interest.

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