Research Article

Determination of Plant Constituents in Fractions of *Cordia obliqua* Willd. Leaf Methanol Extract using GC-MS Analysis

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ABSTRACT

Cordia obliqua Willd. is an important medicinal plant of family Boraginaceae. It is commonly known as Clammy Cherry. It is a deciduous tree and found throughout Himalayan region in India. Traditionally, it possesses a number of medicinal activities like diuretic, expectorant, antipyretic, anthelmintic and maturant. The current work is aimed to investigate chemical constituents present in fractions of leaf methanol extract of *Cordia obliqua* plant using GC-MS analysis. Two fractions of methanol extract (CO1 & CO3) were studied and about 30 components were detected in each sample. Highest peak area was observed for moncyclic sesquiterpene alcohol α-Bisabolol oxide b at RT 20.84 in CO1 and for o-methyl-d-glucose at RT 16.53 in CO3. The important constituents detected were Amino acids, Oleic acid, Oleanolic acid, Sesquiterpene lactone, trietrpenoids, various steroids, galactopyranoside, Hexadecanoic acid, Phthalic acid, Azafrin, Decatrienoic acid, Docasenamide, Morphinan and carotenoids. Among these Morphinan is an opoid alkaloid and good psychoactive drug. Other constituents are also medicinally important and many have reported biological activities. Thus GC-MS analysis revealed the existence of various types of constituents in *Cordia obliqua* leaf methanol extract fraction which confirm the application of this plant for a number of medicinal activities.

Keywords: *Cordia obliqua* Willd., Boraginaceae, GC-MS analysis, Indole alkaloid, Triterpenoids.

INTRODUCTION

Plants are always recognized as powerful and rich sources of various chemical constituents that are identified as potent medicinal agents. So, over the years, World Health Organization (WHO) has supported use of traditional medicines as safe remedies for a number of ailments[1].

The knowledge of chemical constituents present in plant is desirable not only for the discovery of new therapeutic agents but also this information is also of great value in disclosing the new sources of economically important phytocompounds for synthesis of complex chemical substances and for discovering actual significance of traditional remedies[2].

Cordia obliqua Willd. (Clammy Cherry, Lasora) belongs to Boraginaceae family. In India, it is found throughout Himalayan region up to an elevation of 1470 meters and also found in other countries like New Guinea, Philippines, Zava, Formosa and Hainan. It has good medicinal value and show activities like diuretic, expectorant, antipyretic, anthelmintic, maturant and analgesic. The plant fruits are very sweet when ripen and can be used as vegetable and pickle in raw form. The plant contains various constituents like Phenolics, Triterpenes, Pyrrolizidine Alkaloids, Tannins and Flavonoids. From parts of plant like seeds, fruits, stem bark and roots, some constituents are isolated as Lupeol, Octacosanol, Alpha-amyrin, Hentricontanol, Beta-sitosterol, Taxifolin-3,5-dirhamnoside and

Hesperitin-7-rhamnoside, but no chemical examination report on leaf part is present till[3]. So this work is designed to investigate the chemical constituents present in leaf methanol extract because as per previous study, methanol extract is rich in plant constituents and has shown better antioxidant effect[4]. For this analysis, Gas Chromatography with Mass Spectrometry (GC-MS) method was selected because it is one of the good analytical technique for finger print analysis of various chemical and natural compounds like volatile oil, lipids, fatty acids, alkaloids, sugar and steroids. GC-MS provides advantage of both chromatography (a separation method) and mass spectrometry as molecule identification method[5].

MATERIALS AND METHODS

Plant material

The plant leaves were procured from Jammu. These were authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist at Raw Material Herbarium and Museum (RHMD), Council of Scientific and Industrial Research-National Institute of Science and Information Resources (CSIR-NISCAIR), New Delhi (India), with the reference number NISCAIR/RHMD/Consult/-2014/2383-163. A plant sample was also submitted in Pharmacognosy department herbarium of ASBASJSM College of pharmacy, Bela (Ropar) for future reference.
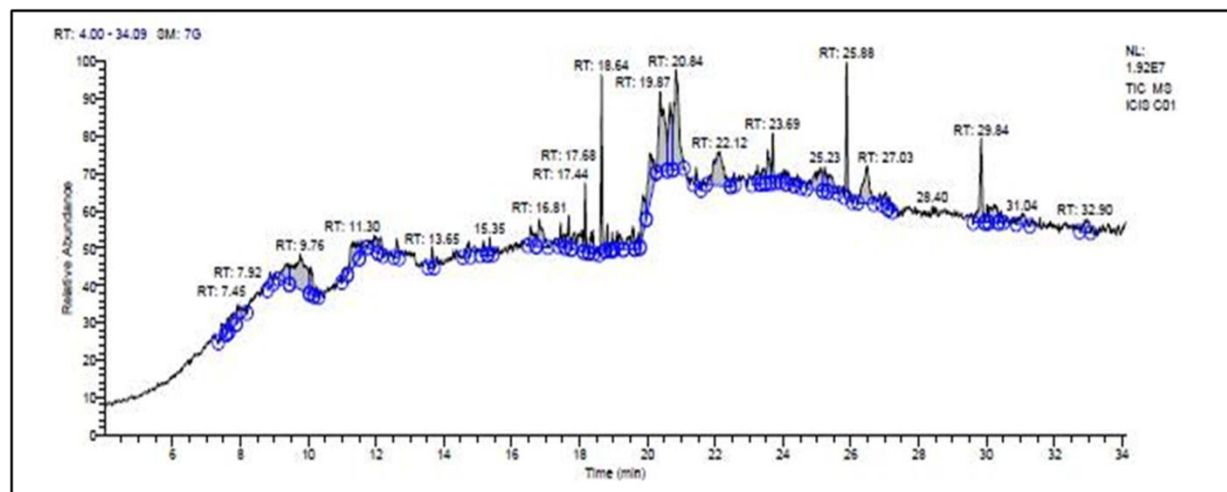
Extraction method

Figure 1: GC-MS Analysis of CO1 Fraction of *Cordia obliqua* Leaf Methanol Extract.

The leaves (about 1 Kg) were dried under shade and coarsely powdered with help of grinder. Then extraction of leaf was performed successively using soxhlet apparatus (continuous hot percolation) with a number of solvents in order of increasing polarity such as Hexane, Chloroform, Methanol and water. All the four extracts were concentrated using Rotary vacuum evaporator separately and kept in desiccator. The methanol extract yield was found 1.2%w/w and it was non sticky and dark Green in color. As per the previous study, methanol extract contains maximum number of plant constituents as well as maximum total phenol, total flavonoid content and anti-oxidant activity. So leaf methanol extract was selected for study of determination of chemical constituents by GC-MS.

GC-MS analysis of Cordia obliqua leaf methanol extract
To investigate the various phyto-constituents present in leaf methanol extract, solvent fractionation was performed. About 5g of methanol extract was taken and dissolved in little amount of water. Then treated with various solvents successively in a separating funnel *viz.* Hexane, Chloroform, Ethyl acetate and n-butanol. All fractions (total 5) obtained were concentrated using Rotary vacuum evaporator. These were weighed and the yield was 1.88%w/w, 17.6%w/w, 16.3%w/w, 24.92%w/w and 32.2%w/w respectively. These all were studied by Thin layer chromatography using Ethyl acetate, Methanol and water(77:15:8) solvent system and detected by spraying of Anisaldehyde- sulphuric acid reagent and then heating at 100°C in hot air oven for 5-10 minutes[6]. In n-butanol and remaining methanol fraction, 4-5 spots were observed.
According to result of thin layer chromatography study, sample of n-butanol and remaining methanol fraction (CO1 and CO3 respectively) were sent for GC-MS analysis at SAIF, Punjab University, Chandigarh. The instrument used was Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. Helium gas was used as a carrier at flow rate of 1ml/min. Sample volume 1μl was injected. Split/Splitless injector was used with injector temperature 250°C. The ion source was

maintained at 230°C temperature. The oven temperature was maintained initially at 80°C for 2 min and then programmed to increase up to 280°C at a rate of 5°C/min and ending with a isothermal for 5 min at 280°C. Total scans were taken 3583 and 3584 with total run time 30.09 and 30.10min respectively for CO1 and CO3. Mass range was detected 50-700 m/z for both samples.
Identification of components
To identify the components present in leaf extract fractions, their retention time and mass spectra fragments were compared with the data stored in the computer library and from the data available in National Institute of Standards and Technology (NIST) library[7]. This helps in determination of chemical nature of compounds present in plant which may support the plant's commercial and traditional use as a herbal medicine.

RESULT
The GC-MS study of CO1 and CO3 has shown a number of chemical constituents. The chemical nature of compounds was identified on the basis of molecular formula, retention time and molecular weight. Among these some important constituents are summarized in the following table containing the compound name, retention time (RT), Molecular formula, Area% and compound nature of CO1 and CO3 (Table1,2 and Figure I,II).
This study tells a variety of plant constituents present in leaf methanol extract like Indole alkaloids, saturated and unsaturated fatty acids, lactones, sesquiterpenes, steroids, pentacyclic triterpenoids, Amino acids, amides, esters, Phenanthrene derivatives, hydrocarbons, Carotenoids, xanthophylls, Macrolides and Prostaglandins. It also supports the already isolated constituents from different plants of *Cordia* genus[8,9]. This is the first study reported on chemical constituent determination of *Cordia obliqua* Willd. leaf. The presence of these constituents also confirms the pharmacological activity of leaf extract because many constituents have reported biological activity as given in table 3. It also supports the activity of

Table 1: Chemical Constituents Analyzed By GC-MS In CO1.

S. No.	Compound name	RT	Molecular Formula	Area %	Nature of compound
1.	Azuleno[4,5-b]furan-2(3H)-one	7.45	$C_{17}H_{24}O_6$	1.07	Lactone
2.	Olean-12-ene-3,16,21,22,23,28-hexol	7.45	$C_{30}H_{50}O_6$	1.07	Triterpenoid
3.	3à,4á-Dihydroxy-1,5,7à(H),6á(H)-guai-10(15),11(13)-dien-6,12-olide	7.61	$C_{15}H_{20}O_4$	0.42	Sesquiterpene lactone
4.	Agaricic acid	7.77	$C_{22}H_{40}O_7$	1.23	Fatty acid
5.	2-Secoandrosta-1,6-diene-17,19-diol,	7.77	$C_{25}H_{33}NO_4$	1.23	Steroid
6.	7,8-Epoxylanostan-11-ol	7.92	$C_{32}H_{54}O_4$	1.37	Steroid
7.	1-acetyl-20à-hydroxy-16-methylene-Olean-12-en-28-oic acid	7.92	$C_{21}H_{26}N_2O_2$	1.37	Triterpenoid
8.	Pregn-4-ene-3,20-dione	8.87	$C_{23}H_{36}N_2O_4$	0.67	Steroid
9.	8,14-Seco-3,19-epoxyandrostane-8,14-dione	8.87	$C_{24}H_{36}O_6$	0.67	Steroid
10.	Oleic acid, eicosyl ester	11.30	$C_{38}H_{74}O_2$	2.65	Fatty acid
11.	3,12-Oleandione	12.13	$C_{30}H_{48}O_2$	0.80	Pentacyclic Triterpenoid
12.	Olean-12-en-28-oic acid	14.75	$C_{30}H_{48}O_2$	0.80	Pentacyclic Triterpenoid acid
13.	Corticosterone	15.17	$C_{21}H_{30}O_4$	0.68	Steroid
14.	Allo-cassaic acid methyl ester	15.17	$C_{21}H_{32}O_4$	0.68	Phenanthrene derivative
15.	Psi.-Carotene	15.17	$C_{40}H_{56}O_2$	0.68	Carotene
16.	Lup-20(29)-en-3-ol,acetate, (3a)	15.35	$C_{32}H_{52}O_2$	0.46	Pentacyclic triterpenoid
17.	9,19-Cyclochloestene-3,7-diol,4,14-di methyl-3-acetate	15.35	$C_{31}H_{52}O_3$	0.46	Steroid
18.	4,5-Secoakuammilan-17-oic acid (Lonicerine)	15.35	$C_{21}H_{26}N_2O_4$	0.46	Aspidophylline-type alkaloids (Indole alkaloids)
19.	17-Pentatriacontene	16.81	$C_{35}H_{70}$	1.95	Hydrocarbon
20.	Octadecanoic acid (Stearic acid)	16.81	$C_{19}H_{38}O_3$	1.95	Saturated fatty acid
21.	18,19-Secoyohimban-19-oic acid	17.44	$C_{21}H_{24}N_2O_3$	1.04	Indole-Quinolizine alkaloid
22.	Phthalic acid	17.68	$C_{26}H_{42}O_4$	1.22	Aromatic dicarboxylic acid
23.	Dibutylphthalate	18.17	$C_{16}H_{22}O_4$	1.34	Aromatic acid ester
24.	Anobin	18.96	$C_{15}H_{20}O_5$	0.35	Sesquiterpene Lactone
25.	17.alfa.,21á-28,30-Bisnorhopane	20.38	$C_{28}H_{48}$	6.78	Pentacyclic triterpenes of Hopane type
26.	17-(4-hydroxy-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydrocyclopenta[a]phenanthryl ester	23.55	$C_{33}H_{56}O_4$	1.42	Steroid
27.	Olean-12-ene-3,16,21,22,28-pentol, (3á,16à,21á,22à	24.03	$C_{30}H_{50}O_5$	0.70	Pentacyclic triterpenoid
28.	9,12-Octadecatrienoic acid	24.14	$C_{27}H_{52}O_4Si_2$	0.68	Unsaturated fatty acid
29.	Glycine	24.14	$C_2H_5NO_2$	0.68	Amino acid
30.	Gamabufotalin	25.11	$C_{24}H_{34}O_5$	2.75	Bufadienolide
31.	Betamethasone acetate	25.37	$C_{24}H_{31}FO_6$	1.36	Corticosteroid

leaf methanol extract as analgesic, anti-inflammatory and antipyretic drug.

DISCUSSION

GC-MS is a good technique to identify variety of constituents of volatile matter, long chain & branched chain hydrocarbons, acids, alcohols, esters, amides etc and also other variety of natural components. In GC-MS analysis of CO1 and CO3, various types of constituents were detected at different retention time. The constituents are separated out according to their mass to charge ratio at particular retention time[7]. GC-MS analysis of CO1 revealed the presence of a number of constituents and highest peak area was detected at RT 20.84 for moncyclic sesquiterpene alcohol α-Bisabolol oxide b and lowest peak area at RT 18.96. Many important constituents were found out such as Oleanolic acid, Agaricic acid, Bisnorhopane, sesquiterpene lactone, Oleic acid, Corticosterone, Lupeol acetate, Indole alkaloids, Pentriacontene, Octadecanoic acid, Phthalic acid, Carotenes and Xanthophylls. Many of these constituents have good pharmacological importance and Lupeol in form of glycoside has already been isolated from *Cordia obliqua* root part. GC-MS analysis of CO3 also revealed the presence of many important constituents

Table 2: Chemical Constituents Analyzed By gc-Ms In Co3.

S. No.	Compound name	RT	Molecular formula	Area %	Nature of compound
1.	L-Lysine	8.27	$C_{53}H_{72}N_8O_9$	0.49	α-amino acid
2.	Apo-carotenoic acid	8.27	$C_{36}H_{48}O_2$	0.49	Carotenoid
3.	Olean-12-ene-3,16,21,22,28-pentol-21-(2-methyl-2-butenoate), [3á,16à,21á(Z),22à]	8.95	$C_{35}H_{56}O_6$	1.36	Triterpenoid derivative
4.	3à,4á-Dihydroxy-1,5,7à(H),6á(H)-guai-10(15),11(13)-dien-6,12-olide	9.29	$C_{15}H_{20}O_4$	3.62	Sesquiterpene lactone
5.	Olean-12-ene-3,16,21,22,23,28-hexol	9.88	$C_{30}H_{50}O_6$	0.54	Triterpenoid derivative
6.	Olean-12-ene-3,16,21,22,28-pentol	11.76	$C_{35}H_{56}O_6$	1.66	Triterpenoid derivative
7.	2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	15.15	$C_{27}H_{42}O_4$	1.00	Steroid derivative
8.	9,10-Secocholesta-5,7,10(19)-triene-1,3diol	15.45	$C_{30}H_{52}O_3Si$	2.75	Steroid
9.	3-O-Methyl-d-glucose	16.53	$C_7H_{14}O_6$	14.07	Sugar
10.	MyoInositol, -4-C-methyl	16.53	$C_7H_{14}O_6$	14.07	Alcohol of cyclohexane
11.	3-ethyl-5-(2-ehylbutyl)-Hexadecanoic acid	16.99	$C_{37}H_{72}O_4$	0.23	Fatty acid
12.	Phthalic acid	18.17	$C_{22}H_{34}O_4$	1.03	Aromatic dicarboxylic acid
13.	Carotene,	18.38	$C_{42}H_{64}O_2$	1.02	Carotenoid
14.	Dibutyl phthalate	18.65	$C_{16}H_{22}O_4$	10.32	Aromatic acid ester
15.	8,14-Seco-3,19-epoxyandrostane-8,14-dione	19.20	$C_{24}H_{36}O_6$	0.29	Steroid
16.	7,8-epoxylanostan-11-ol	23.56	$C_{32}H_{54}O_4$	3.03	Steroid
17.	3-acetoxyEthyliso-allocholate	23.56	$C_{26}H_{44}O_5$	3.03	Steroid
18.	2,4,6-Decatrienoic acid	23.69	$C_{35}H_{46}O_8$	1.72	Unsaturated fatty acid
19.	8,14-Seco-3,19-epoxyandrostane-8,14-dione	24.02	$C_{24}H_{36}O_6$	3.25	Steroid
20.	13-Docosenamide	25.88	$C_{22}H_{43}NO$	2.46	Amide of Docosenoic acid (Fatty acid)
21.	1,8,15,22-Tetraaza-2,7,16,21-cyclooctacosanetetrone 8H-Azecino[5,4b]indol-8-one,	28.14	$C_{24}H_{44}N_4O_4$	1.17	Indole alkaloids
22.	Milbemycin B	28.27	$C_{33}H_{47}ClO_7$	1.51	Macrolide, antiparasitic
23.	Morphinan	28.27	$C_{16}H_{21}N$	1.51	Opiate alkaloid, Psychoactive drug
24.	Pregn-4-ene-3,20-dione, 17,21-dihydroxy, bis(O-methyloxime)	28.39	$C_{23}H_{36}N_2O_4$	0.85	Corticosteroid
25.	17-acetoxy-3á-methoxy-4,4-dimethyl-4'-Apo-á,.psi.-carotenoicacid	28.46	$C_{35}H_{46}O_2$	1.85	Carotenoid
26.	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	28.80	$C_{28}H_{40}O_{10}$	1.77	Ingol diterpene derivative
27.	(5á)Pregnane-3,20á-diol	30.99	$C_{28}H_{43}NO_6$	0.37	Steroid
28.	8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-6b-hydroxy-3a-methoxy-1,1,5,7-tetramethyl-,[1aR-(1aà,1bá,1cà,2aà,3aà,6aà,6bà,7à,8á,8aà)]-Prosta-5,13-dien-1-oic acid	30.99	$C_{27}H_{36}O_{10}$	0.37	Prostaglandin

and highest peak area was detected at RT 16.53 for 0-methyl-d-glucose and lowest peak area at RT 13.17 and 30.99. The important constituents detected were Amino acids, Oleic acid, Oleanolic acid, Sesquiterpene lactone, trietrpenoids, various steroids, galactopyranoside, Hexadecanoic acid, Phthalic acid, Azafrin, Decatrienoic acid, Docasenamide, Morphinan and carotenoids. Among these Morphinan is opoid alkaloid and good psychoactive drug. Other constituents are also medicinally important. Thus GC-MS analysis revealed the existence of various

Table 3: Reported Medicinal Activities of Various Plant Constituents Analyzed by GC-MS of *Cordia obliqua* Willd. Leaf Methanol Extract Fractions.

S. No.	Chemical Constituent	Activities reported
1.	Oleanolic acid and derivatives	Treatment of diabetes and obesity[10]
2.	Dihydroxy guaidien-6,12-olide (Sesquiterpene Lactone)	Antiproliferative and apoptotic[11]
3.	Plant steroids	Anabolic and anti-inflammatory[12,13]
4.	Octadecanoic acid, Oleic acid and other fatty acids	Management of eye pains and inflammatory conditions from bacterial and fungal infections[14]
5.	18,19-secoyohimban-19-oic acid	Cardiovascular and spermatogenic effect[14]
6.	Gamabufotalin	Anticancer and anti-inflammatory[15]
7.	Pregn-4-ene-3,20-dione (Progesterone)	Hormone replacement therapy
8.	Corticosterone	Regulation of energy, immune reactions and stress responses
9.	Carotene	Antioxidant
10.	Lup-20(29)-en-3-ol,acetate	Antioxidant and anti-inflammatory[16]
11.	Glycine	In treatment of metabolic disorders and stroke
12.	Betamethasone acetate	Anti-inflammatory, analgesic and in skin problems
13.	Bisnorhopane	Anti MRSA (Methicillin Resistant Staphylococcus aureus)activity
14.	Myoinositol	Insulin sensitizer, In diabetic nerve pain, insomnia, high cholesterol Schizophrenia
15.	Milbemycin	Antiparasitic
16.	Morphinan	Opiate alkaloid, Psychoactive drug[17]
17.	3,8,12-tri-o-acetylingol	Antibacterial
18.	8,14-Seco-3,19-epoxyandrostane-8,14-dione	Anticancer[18]
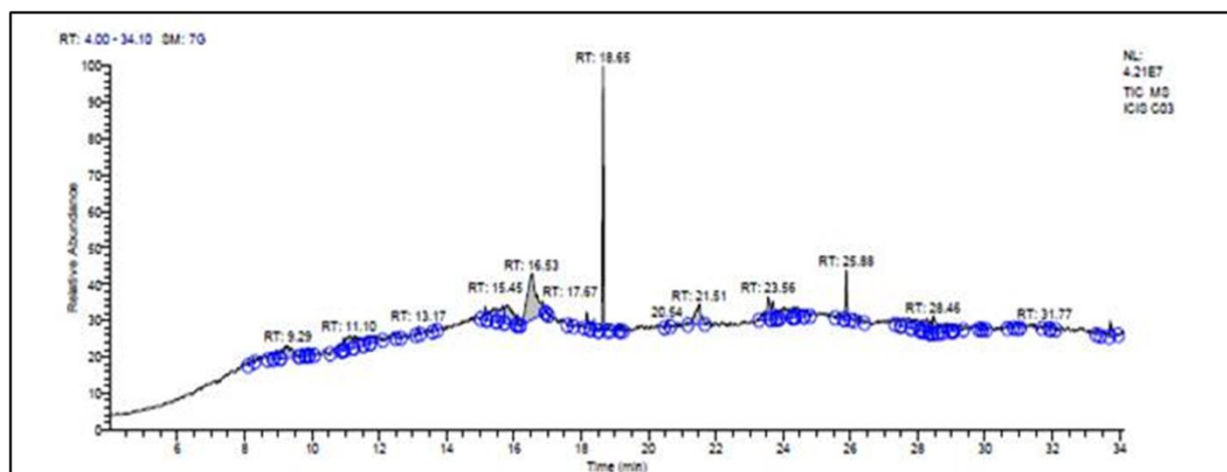
Figure 2: GC-MS Analysis Of Co3 Fraction Of *Cordia Obliqua* Leaf Methanol Extract.

types of constituents in *Cordia obliqua* leaf methanol extract fraction which confirm the application of this plant for a number of medicinal activities. Further detail phytochemistry and isolation of individual constituent may add the knowledge about the characteristic structure and particular mechanism of action of the drug.

CONCLUSION

In the conclusion, GC-MS analysis revealed the presence of many important chemical constituents which support the already determined constituents from *Cordia* genus. It is the first study related with determination of chemical nature of *Cordia obliqua* leaf methanol extract and it also suggests the medicinal importance of *Cordia obliqua* leaf for a number of ailments. Further, the isolation of active phyto-constituents from plant will help in understanding the mechanism of action and also identification of lead molecules of clinical utility.

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