

Phytochemical Analysis Revealing the Presence of Two New Compounds from the Latex of *Calotropis Procera* (Ait.) R.Br.

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Abstract *Calotropis procera* (Ait.) R.Br. (Asclepiadaceae) is a well known Indian medicinal plant. Its latex constituents having high calorific value because of its rich hydrocarbons. Moreover it was traditionally applied for tanninizing leather in India. Several attempts have been made in the past to establish it as a biofuel crop. In the present study the major phyto-constituents from the dried latex of *C. procera* are identified through GC-MS analysis. Acetone extract derived from the dried latex of *C. procera* was further partitioned between n-hexane and ethanol (10:90). The n-hexane layer was separated, dried and re-dissolved in Acetone. The un-dissolved part was dissolved in n-Hexane:Methanol (9:1) combination. Both the fractions were subjected for GC-MS analysis and nine hydrocarbon compounds have been identified from the latex, out of which 2,6 dimethyl tetra-1,5-decaene and 3, 7,11-Trimethyl-2,6,10,12-pentadecatrien-1-ol are newly reported from latex of *C. procera*.

Keywords *Calotropis procera*, Asclepiadaceae, medicinal plant, bio-energy, latex, phytochemistry, hydrocarbons, GC-MS

1. Introduction

Calotropis procera belongs to the family Asclepiadaceae and is a well known Indian medicinal plant locally known as "Akado". Medicinally important parts of this plant are flower, terminal leaf pairs, root with root bark and latex. Out of that the most significant part which drew attention of researchers is latex because of its medicinal properties, economic use for cheese and rubber production. Latex contain majority of hydrocarbon contents[1]. Kumar et al.[2] have established this plant as a source of bio-energy. The plant also shows good antibacterial activity[3]. Therefore, in the present study the major latex constituents were separated and identified through GC-MS analysis.

2. Material and Methods

2.1. Plant Material

Latex of *C. procera* was collected from the arid area of Siddhpur, North Gujarat, India. The latex was dried in oven at 42°C to obtain 20% dry powder.

2.2. Extraction and Characterization

The dry latex powder was extracted with acetone in Soxhlet apparatus for 12hrs. The dried acetone extract was partitioned between n-hexane and 90% ethanol combined in 1:5 ratios. The layer of n-hexane was separated and evaporated to get oily mixture of hydrocarbons in liquid form. All hydrocarbons were separated through TLC in solvent system n-hexane: di-ethyl ether: acetic acid (80:20:1). The resolved bands compared with the standard compounds oleic acid, triolein and β -sitosterol. The TLC plate developed with the spray of vanillin-sulphuric acid[4]. The dried n-hexane layer was again re-dissolved in acetone and remaining part was dissolved in n - hexane:methanol (9:1) in warm condition (60°C). Both the fractions of n-hexane extract were analyzed by GC-MS. Solvents used for extraction and purification were from MERCK company.

2.3. Parameters of GC-MS Analysis

GC-MS model: Perkin Elmer Autosystem XL with Turbomass, Column type: PE-5MS, Column Material: 5%

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Published online at <http://journal.sapub.org/plant>

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Phenyl polysiloxane, Column Length: 30 meters, Column inner diameter: 0.250 mm, Flow rate (N₂): 1 ml/min, Temperature of injector: 230°C, Temperature of detector: 280°C, Temperature of source: 280°C, Temperature of transfer: 280°C, Programming rate: Starting from 78°C for 5min. Increasing temperature with rate 10°C/min up to 280°C and hold for 20min. Retention time: 45min

3. Result and Discussion

Latex of *Calotropis procera* is well known for cardiac glycosides and hydrocarbons[5]. The reported cardiac glycosides were Calotropogenin[6], Calotropin[7], Calotoxin, Uscharin and Calactin[8] with identification of some hydrocarbon derivatives like Linoleic acid, Oleic acid and Palmitic acid[9].

The maximum portion of dry latex of *C. procera* extracted by acetone was 54%. During initial screening the other solvents tried were n-hexane, petroleum ether, chloroform and dimethyl sulphoxide but none of them could extract as much large part as acetone. Majority of the latex is composed of hydrocarbons therefore n-hexane part of acetone extract was concentrated for separation and identification. The presence of three marker compounds oleic acid (Rf: 0.51), triolein (Rf: 0.89) and β -sitosterol (Rf: 0.30) were confirmed through TLC.

Major nine compounds of n-hexane fraction are identified from both the fractions of n-hexane as described in experimental section through GC-MS. The identified compounds are checked with the available references and the two compounds are not found to be reported anywhere from *C. procera* are 2,6 dimethyl tetra-1,5-decaene and 3, 7,11-Trimethyl-2,6,10,12-pentadecatrien-1-ol, they are reported as the new phytoconstituents from *C. procera*. The four another compounds are excluded from the study are Ethyl phthalate, Di-n-Propyl phthalate, Phthalic acid diisobutyl ester and Phthalic acid diisooheptyl ester as the contaminants. Their details of retention time and relative percentage of the corresponding latex components are given in Tables-1, 2 & 3.

The electron impact mass spectral (EI-MS) fragmentations of the major nine prominent phytochemicals from the latex of *C. procera* are narrated as under:

Table 1. Major chemical constitution from latex of *C. procera*

Compound	RT (min)	Conc. (%)	Identified compound
1.	3.569	1.16	4-hydroxy-4-methylpentan-2-one
2.	4.467	1.09	2,3,4-trimethylhexane
3.	7.015	3.71	Decane
4.	12.772	1.16	n-Pentadecane
5.	16.861	1.93	2,6 dimethyl tetra-1,5-decaene
6.	24.928	1.17	n-Eicosane
7.	32.371	5.98	3, 7,11-Trimethyl-2,6,10,12-pentadecatrien-1-ol
*8.	32.390	0.80	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene
9.	35.341	17.54	1,3,5-Triisopropylbenzene

Table 2. Major chemical constitution from latex of *C. procera*

Compound	M.W. (g/mol)	Molecular Formula	CAS Number	Boiling Point (°C)
1.	116.16	C ₆ H ₁₂ O ₂	000123-42-2	116
2.	128.26	C ₉ H ₂₀	000921-47-1	140
3.	142.28	C ₁₀ H ₂₂	000124-18-5	174
4.	212.42	C ₁₅ H ₃₂	000629-62-9	270
5.	220.40	C ₁₆ H ₂₈	----	--
6.	282.56	C ₂₀ H ₄₂	000112-95-8	220
7.	262.44	C ₁₈ H ₃₀ O	----	--
*8.	410.72	C ₃₀ H ₅₀	000111-02-4	285
9.	204.35	C ₁₅ H ₂₄	000717-74-8	232

*Peak No 8 from n-hexane:methanol (9:1) soluble part of n-hexane layer of acetone extract from dry *Calotropis procera* latex.

Table 3. Structural configuration of major chemical constitution from latex of *Calotropis procera*

Compound	RT (min)	Structural Formula
1.	3.569	
2.	4.467	
3.	7.015	
4.	12.772	
5.	16.861	
6.	24.928	
7.	32.371	
8.	32.390	
9.	35.341	

Compound 1 (RT-3.569): Comparison of fragments ions in mass Spectra (MS) of peak (RT 3.569) identified as 4-hydroxy-4-methylpentan-2-one. EI/MS fragment ions m/z (percentage base peak): [M⁺] 116, [M⁺-C₂H₃O] 43(100); [M⁺-C₃H₇O] 58(22); [M⁺-C₄H₁₀O] 74(3); [M⁺-C₃H₅O] 88(3); [M⁺-C₆H₁₀O] 98(5); [M⁺-C₅H₉O₂] 101(15).

Compound 2 (RT-4.467): Comparison of fragments ions in mass Spectra (MS) of peak (RT 4.467) identified as 2,3,4-trimethylhexane. EI/MS fragment ions m/z (percentage base peak): [M⁺] 128; [M⁺-CH₃]; 113(3); [M⁺-C₂H₅] 99(10); [M⁺-C₄H₉]; 71(33); [M⁺-C₆H₁₃] 43(100); [M⁺-C₅H₁₁] 57 (90).

Compound 3 (RT-7.015): Comparison of fragments ions in mass Spectra (MS) of peak (RT 7.015) identified as Decane. EI/MS fragment ions m/z (percentage base peak): [M⁺] 142; [M⁺-CH₃] 127(3); [M⁺-C₂H₅] 113(5); [M⁺-C₃H₇] 99(5); [M⁺-C₄H₉] 85(30); [M⁺-C₇H₁₆] 43(100); [M⁺-C₆H₁₃]

57(95); [M⁺-C₁₈H₁₇] 29(40).

Compound 4 (RT-12.772): Comparison of fragments ions in mass Spectra (MS) of peak (RT 12.772) identified as n-Pentadecane. EI/MS fragment ions m/z (percentage base peak): [M⁺] 212; [M-CH₃] 197(3); [M⁺-C₂H₅] 183(3); [M⁺-C₃H₇] 169(3); [M⁺-C₄H₉] 155(3); [M⁺-C₁₁H₂₃] 57(100); [M⁺-C₁₂H₂₅] 43(80); [M⁺-C₁₃H₂₇] 29(35).

Compound 6 (RT-24.928): Comparison of fragments ions in mass Spectra (MS) of peak (RT 24.928) identified as n-Eicosane. EI/MS fragment ions m/z (percentage base peak): [M⁺] 282; [M⁺-C₂H₅] 267(3); [M⁺-C₃H₇] 253(3); [M⁺-C₃H₁₁] 225(3); [M⁺-C₁₇H₃₅] 57(100); [M⁺-C₁₈H₃₇] 43(73); [M⁺-C₁₉H₃₉] 29(18).

Compound 7 (RT-32.371): Comparison of fragments ions in mass Spectra (MS) of peak (RT 32.371) identified as 3, 7,11-Trimethyl-2,6,10,12-pentadecatrien-1-ol. EI/MS fragment ions m/z (percentage base peak): [M⁺] 262; [M⁺-OH] 244(3); [M⁺-C₄H₇] 207(5); [M⁺-C₁₂H₁₉] 99(5); [M⁺-CH₃O] 231(5); [M⁺-C₂H₄O] 218(100); [M⁺-C₃H₇O]; 203(85); [M⁺-C₉H₁₅] 139(15); [M⁺-C₁₆H₂₆] 44(15); [M⁺-C₉H₁₅O] 123(10); [M⁺-C₁₀H₁₇O] 109(15); [M⁺-C₁₁H₁₉O] 95(38).

Compound 8 (RT-32.390): Comparison of fragments ions in mass Spectra (MS) of peak (RT 32.390) identified as 2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexane. EI/MS fragment ions m/z (percentage base peak): [M⁺] 410; [M⁺-CH₃] 395(3); [M⁺-C₄H₇] 355(3); [M⁺-C₁₆H₂₇] 191(10); [M⁺-C₂₆H₄₃] 55(23); [M⁺-C₂₅H₄₁] 69(100); [M⁺-C₃H₉] 341(5); [M⁺-C₆H₁₁] 327(5); [M⁺-C₉H₁₅] 287(3).

Compound 9 (RT-35.341): Comparison of fragments ions in mass Spectra (MS) of peak (RT 35.341) identified as 1,3,5-Triisopropylbenzene. EI/MS fragment ions m/z (percentage base peak): [M⁺] 204; [M⁺-CH₃] 189(100); [M⁺-C₂H₆] 174(22); [M⁺-C₃H₇] 161(30); [M⁺-C₁₂H₁₇] 43(63).

Compound 5 (RT-16.861): Comparison of fragments ions in mass Spectra (MS) of peak (RT 16.861) identified as 2,6 dimethyl tetra-1,5-decaene. EI/MS fragment ions m/z (percentage base peak): [M⁺] 220; [M⁺-CH₃] 207(3); [M⁺-C₂H₅] 193(5); [M⁺-C₃H₃] 181(6); [M⁺-C₅H₉] 153(10); [M⁺-C₇H₁₅] 123(100); [M⁺-C₉H₁₅] 99(10); [M⁺-C₁₁H₂₁] 69(15); [M⁺-C₁₄H₂₅] 29(28).

4. Conclusions

Acetone extract of *C. procera* latex investigated through its n-hexane fraction with identification of major nine organic compounds with the report of two new compounds 2,6 dimethyl tetra-1,5-decaene and 3, 7,11-Trimethyl-2,6,10, 12-pentadecatrien-1-ol first time from *C. procera*.

ACKNOWLEDGEMENTS

We are thankful to the Department of Science and Technology (DST), New Delhi for providing financial support to the project "Development and Standardization of Herbal Anti-malarial Drug". The authors are also gratefully acknowledged the encouragement and support of Director, ARIBAS and Charutar Vidya Mandal (CVM), Vallabh Vidyanagar, Gujarat, India.

REFERENCES

- [1] M D Erdman and B A Erdman, *Calotropis procera* as a source of plant hydrocarbons. *Econ. Bot.* 35, 1981, 467-472.
- [2] Kumar, Vijay Rani, Ashwani Kumar and A K Gupta, *Calotropis procera*: A potential bio-energy plant for arid and semi-arid regions. In: Palz, W., J.Spitzer, K.Maniatis, K.Kwant, P.Helm, A.Grassi. (eds) 12th European Biomass conference : Biomass for energy , Industry and climatic protection. WIP Munich. Germany 2002.
- [3] Farzin Parabia, I.L.Kothari, and M.H.Parabia, Antibacterial activity of solvent fractions of crude water decoction of apical twigs and latex of *Calotropis procera* (Ait.) R. Br.". *Natural product radiance*, 2008, 7(1) 30-34.
- [4] Wagner H. and Blatt S. *Plant Drug Analysis*, Second Edition, Springer, 1996.
- [5] I. L. Kothari1, M.H.Parabia, J.H.Patel, Farzin M. Parabia, Smita S. Pathak, Falguni Sheth, Hiren V. Doshi and M.A.Patel, 2010. An Ethnopharmacological Review on *Calotropis* Species printed in Compendium of Bioactive Natural Products Vol. 3: Efficacy, Safety & Clinical Evaluation (Part-2) in the book series, *Comprehensive Bioactive Natural Products* published by *Stadium Press LLC, USA*.
- [6] E Haslam, Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action. *J. Nat. Prod.* 1996, 59, 205-215.
- [7] V E Tyler, *Phytomedicines: Back to the Future*. *J. Nat. Prod.* 1999 62, 1589-1592.
- [8] J N Seiber, C J Nelson, M S Lee, Cardenolides in the latex and leaves of seven *Asclepias* species and *Calotropis procera*. *Phytochemistry* 21, 1982, 2343-2348.
- [9] J A Duke, *Handbook of phytochemical constituents of GRAS herbs and other economic plants*. Boca Raton, FL. CRC Press. (Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992. [Online Database] (URL: <http://www.arsgrin.gov/cgi-bin/duke/farmacy2.pl>).