www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(7): 463-467

www.thepharmajournal.com
Received: 28-05-2020
Accepted: 30-06-2020

Brij Mohan
Natural Product Laboratory, Department of Chemistry, Govt. Science College, Jabalpur, Madhya Pradesh, India

Hari Om Saxena
NWFP section, SFM & AF Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Samiksha Parihar
NWFP section, SFM & AF Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Arun Kakkar
Natural Product Laboratory, Department of Chemistry, Govt. Science College, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Brij Mohan
Natural Product Laboratory, Department of Chemistry, Govt. Science College, Jabalpur, Madhya Pradesh, India

Gas chromatography-mass spectrometry (GC-MS) determination of phytoconstituents from ethanolic and aqua-ethanolic root extracts of *Uraria picta* Desv. (Fabaceae)

Brij Mohan, Hari Om Saxena, Samiksha Parihar and Arun Kakkar

DOI: https://doi.org/10.22271/tpi.2020.v9.i7g.4973

Abstract
This is a first-time study on Gas Chromatography-Mass Spectrometry (GC-MS) determination of phytoconstituents from ethanolic and aqua-ethanolic root extracts of *Uraria picta.* It is a commercially importance species which is utilized in Dashmoolarishta, an established ayurvedic drug of ISM. GC-MS determined two major phytoconstituents in ethanolic extract as N-Capric acid Isopropyl Ester (RT- 21.397, 60.98%), 9-Octadecenoic acid (Z) -, Hexyl Ester (RT- 23.298, 39.02%) and three compounds in aqua-ethanolic extract namely Propanoic acid, 2- Hydroxy-, Pentyl Ester (RT- 11.697, 81.64%), α - D-Mannofuranoside, 1-Nonyl - (RT- 20.28, 7.87%) and 2-Bromopropionic acid, Penta decyl ester (RT- 21.486, 6.77%). Four phytocompounds investigated from roots in the present study have commercial significance.

Keywords: *Uraria picta*, roots, ethanolic and aqua-ethanolic extracts, GC-MS analysis

1. Introduction
Plants have been used since ancient times to heal and cure diseases and to improve health and wellbeing. Despite ancient nature of the tradition, medicinal plants still form the basis of traditional or indigenous health systems and their use is increasing worldwide. According to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol (Julsing *et al.*, 2007) [1]. There are many reports on the use of medicinal plants traditionally by tribals and indigenous people (Ignacimuthu *et al.*, 1998, Natarajan *et al.*, 1999, Rajan *et al.*, 2002, Ayyanar & Iganacimuthu, 2005 and Sandhy *et al.*, 2006) [2-6]. The quality and efficacy of medicinal plants/raw materials depends on their biologically active compounds (Saxena *et al.*, 2016; Joshi & Uniyal, 2008) [7-8] and there is also need to extract and identify the phytoconstituents for therapeutic target (Vuorela *et al.*, 2004) [9]. Gas chromatography coupled to mass spectrometry (GC-MS) has commonly been used for analysis of volatile bioactive compounds. It is a hyphenated technique which couples two analytical techniques GC and MS to a single system of analyzing mixtures of chemical compounds. GC separates the components of the mixture and MS analyzes each of the components separately through fragmentation (Bai *et al.*, 2014; Arora and Kumar, 2017) [10, 11]. *Uraria picta* Desv. (Syn. *Doodia picta* Roxb., *Hedysarum pictum* Jacq., Family- Fabaceae) is commonly known as Prishnaparni or Pithvan and widely distributed throughout India, Bangladesh, Sri Lanka, Tropical Africa, Malay Islands, Philippines, Australia, Africa and almost all parts of Asia (McNeill *et al.*, 2006; Ohashi and Iokawa, 2007) [12, 13]. It is one of the important constituents of "Dashmoolarista", a well-established ayurvedic drug of Indian system of medicine (ISM), where the roots of this plant are principally employed along with the roots of 10 other plants. The drug is used for treating general fatigue, oral sores and several gynecological disorders (Yadav *et al.*, 2009) [14]. Dashmool is also used as basic ingredient in manufacture of over 109 drug formulations (Pathak *et al.*, 2005) [15]. Traditionally, the plant is used as an antidote to the venom of a dangerous Indian snake, *Echis carinata* (Kirtikar and Basu, 1993) [16]. Its roots are being used against cough, chills and fever (Kirtikar and Basu, 1993; Yusuf *et al.*, 1994) [16, 17]. Since the roots of *U. picta* are utilized in in a number of herbal formulations but still not explored much in terms of its chemical constituents. Therefore, the

present investigation dealt with the identification of new phytoconstituents in ethanolic and aqua-ethanolic extracts of *U. picta* roots.

2. Materials and Methods

2.1 Collection of plant materials

U. picta species was collected by following the guidelines of good agricultural and collection practices (GACP) for medicinal plants (Anon, 2003) [18] from the Khandwa region of Madhya Pradesh, India during December month.

2.2 Processing of plant materials

Plant materials were washed thoroughly in running water to remove soil and other foreign particles. Roots were separated and dried in shade. Shade dried material was powdered using pulverizer. The powdered material was utilized for making extracts.

2.3 Preparation of extracts

Powdered sample was subjected to successive extraction with Ethanol and Water: Ethanol (Aqua- alcoholic) (20: 80) and Water (Varghese *et al.,* 2013) [19]. A total of 20g of dried powder was extracted in 250 ml of each solvent in successive manner for 12 hrs. Solvents were evaporated to dryness to yield the respective extracts.

2.4 GC-MS analysis

Ethanolic, aqua-ethanolic and aqueous extracts were subjected to chemical analysis by using GC-MS instrument, Perkin Elmer, USA & Model - Auto system XL with Turbo Mass. Compounds were separated on PE-5MS 30m x 0.250mm x 0.250µm column. Oven temperature was programmed as follows: isothermal temperature of 75 ºC for min and then increased up to 280 ºC at the rate of 10 ºC/ min and held for 15 min. Injection temperature was 250 ºC and injection volume was 1µl. EI source temperature was set as 220 ºC. Helium gas was used as carrier gas at 1 ml/ min flow rate. MW range was set at 22 to 620 amu. Interpretation of mass spectrum of GC-MS was conducted using the database of NIST. The spectrum of investigated components was compared with spectrum of known components stored in NIST. Molecular weight, molecular formula and number of hits were used to identify the name of compounds from NIST.

3. Results and Discussion

GC-MS chromatograms of ethanolic and aqua-ethanolic extracts of *U. picta* roots are given as Fig. 1 and Fig. 2 respectively. No compound was detected in aqueous extract. On comparison of the mass spectra of the phytoconstituents with the NIST library, two compounds were characterized and identified in ethanolic and three phytoconstituents in aqua-alcoholic extracts, which has been represented in Table 1. The biological activity as well as commercial significance of chemical compounds identified in both extracts is given in Table 2. Chemical structures of compounds identified by GC-MS in the extracts are drawn in Fig. 3. In the ethanolic extract, the main compounds were N-Capric acid Isopropyl Ester, a fatty acid ester (60.98%) and 9-Octadecenoic acid (Z)-, Hexyl Ester, a fatty acid ester (39.02%). The major compounds identified in aqua- alcoholic extract were Propanoic acid, 2- Hydroxy-, Pentyl Ester, short chain fatty acid ester (81.64%), α-D-Mannofuranoside, 1-Nonyl –, Derivative of Monosaccharide (7.87%) and 2-Bromopropionic acid, Pentadecyl ester, fatty acid ester (6.77%). N-Capric acid Isopropyl Ester and Propanoic acid, 2- Hydroxy-, Pentyl Ester have been reported to be utilized as flavor and fragrance agents (Anon, 2020) [20, 22]. 9-Octadecenoic acid (Z)-, Hexyl Ester is a food additive and flavoring agent (Anon, 2020) [21]. 2-Bromopropionic acid, Pentadecyl ester has been reported to have antibacterial activity (Kumar *et al.,* 2011) [23]. No activity was reported in 1-Nonyl - α-D-Mannofuranoside phytocompound.

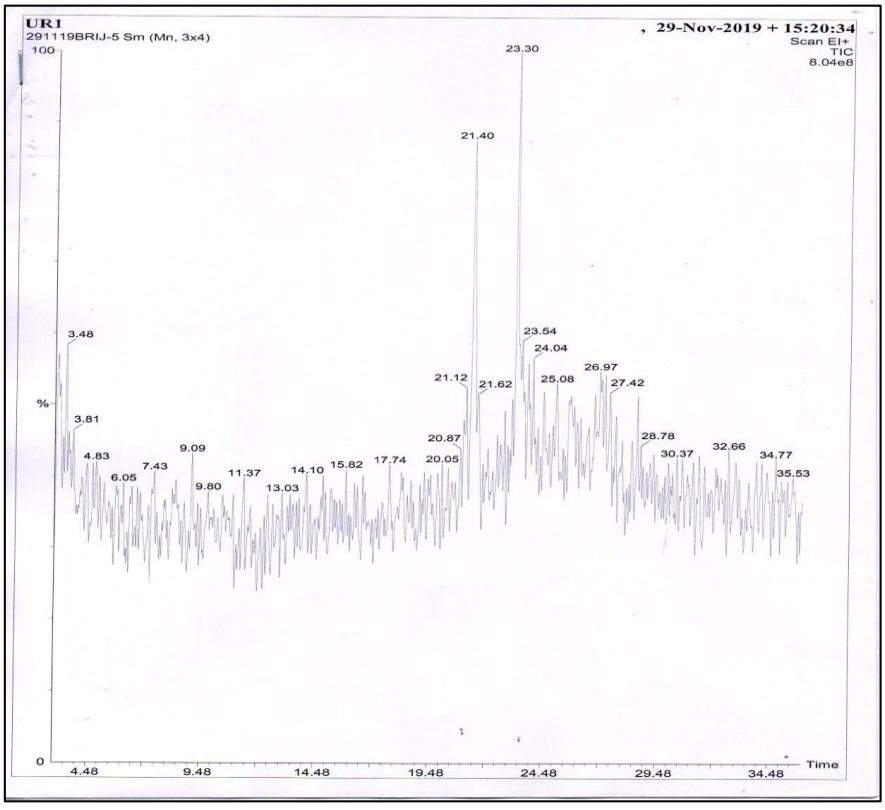


Fig 1: GC-MS chromatogram of ethanolic extract of *U. picta* root

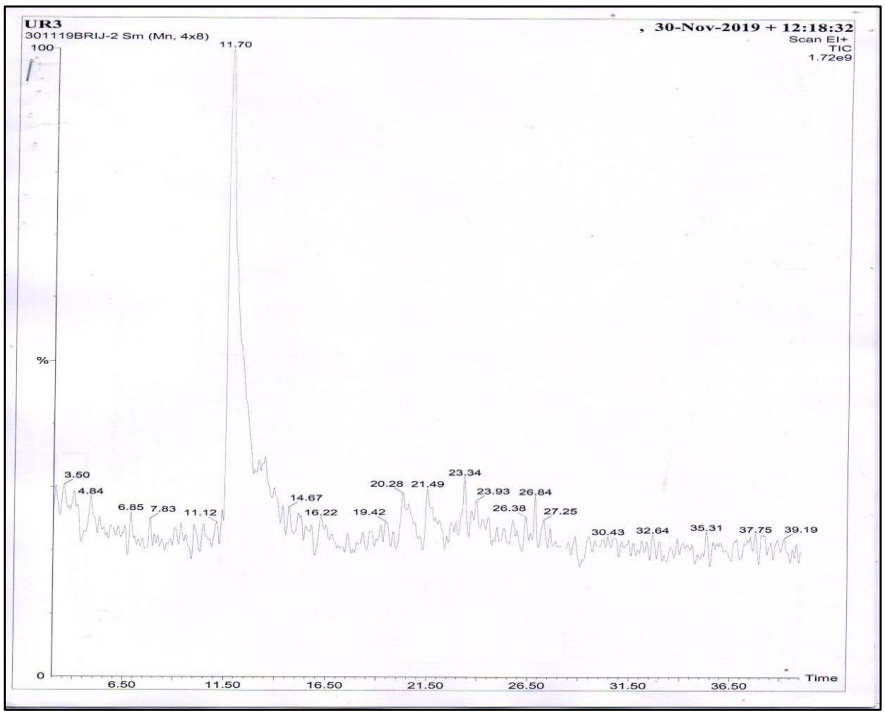


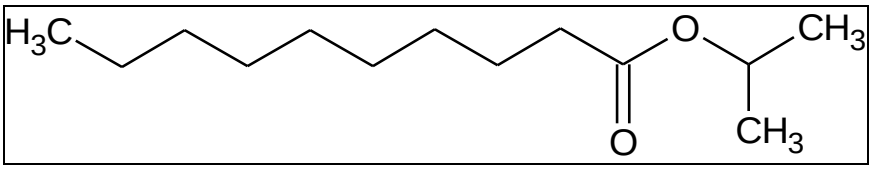
Fig 2: GC-MS chromatogram of aqua-ethanolic extract of *U. picta* root

Table 1: Phytocompounds identified in ethanolic and aqua- ethanolic (20:80) extracts by GC-MS

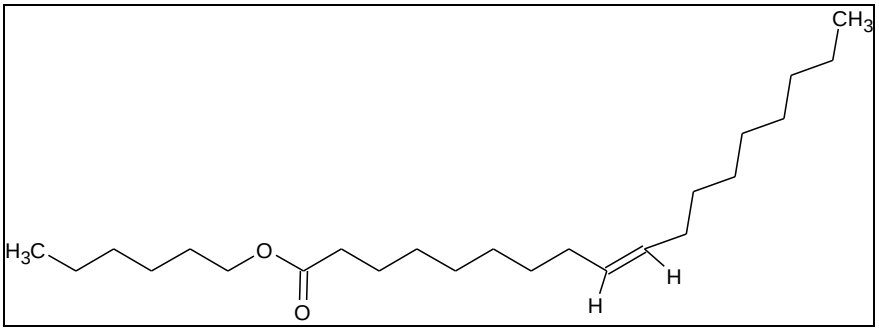
Extracts	Name of phytocompounds	Molecular Formula	Molecular Weight	Exact Mass	RT (min)	Area %
Ethanolic	N-Capric acid Isopropyl Ester	$C_{13}H_{26}O_2$	214	214.34	21.397	60.98
	9-Octadecenoic acid (Z) -, Hexyl Ester or Oleic acid, hexyl ester or Hexyl oleate	$C_{24}H_{46}O_2$	366	366.6	23.298	39.02
Aqua- ethanolic (20:80)	Propanoic acid, 2- Hydroxy-, Pentyl Ester	$C_8H_{16}O_3$	160	160.21	11.697	81.64
	α - D-Mannofuranoside, 1-Nonyl -	$C_{15}H_{30}O_6$	306	306.39	20.28	7.87
	2-Bromopropionic acid, Penta decyl ester	$C_{18}H_{35}O_2Br$	362	363.37	21.486	6.77

Table 2: Bioactivity/ importance of phytocomponents identified in ethanolic and aqua- ethanolic (20:80) extracts by GC-MS

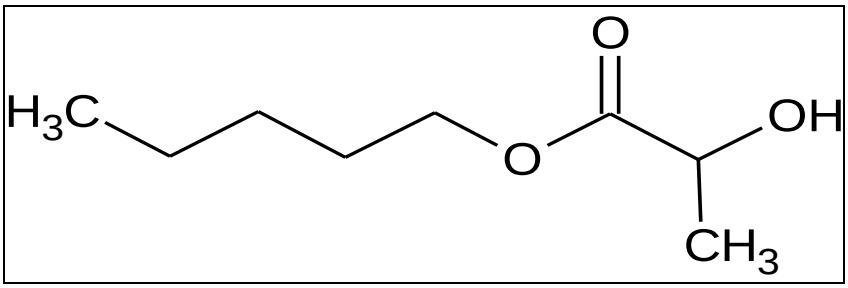
S. No.	Name of compound	Nature of compound	Biological Activity/ Significance
1.	N-Capric acid Isopropyl Ester	Fatty acid ester	Flavor and fragrance agents (Anon, 2020) [20]
2.	9-Octadecenoic acid (Z)-, Hexyl Ester	Fatty acid ester	Food additives and flavoring Agents (Anon, 2020) [21]
3.	Propanoic acid, 2- Hydroxy-, Pentyl Ester	Short chain fatty acid ester	Flavor and fragrance agents (Anon, 2020) [22]
4.	α-D-Mannofuranoside, 1-Nonyl -	Derivative of Monosaccharide	No activity detected
5.	2-Bromopropionic acid, Pentadecyl ester	Fatty acid ester	Antibacterial activity (Kumar *et al.*, 2011) [23]



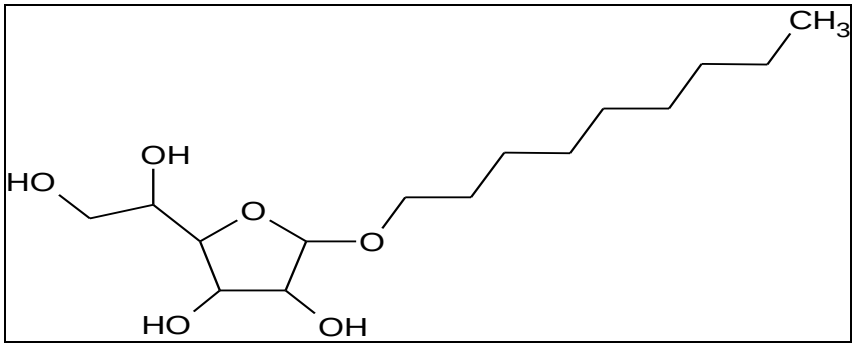
(a)



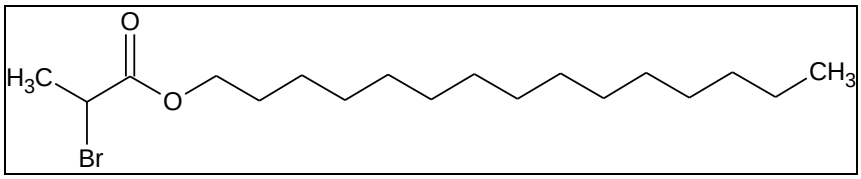
(b)



(c)



(d)



(e)

Fig 3: Chemical structures of phytocompounds identified by GC-MS (i) ethanolic extract (a) N-Capric acid Isopropyl Ester (b) 9-Octadecenoic acid (Z)-, Hexyl Ester (ii) Aqua-ethanolic extract (c) Propanoic acid, 2- Hydroxy-, Pentyl Ester (d) α-D-Mannofuranoside, 1-Nonyl – (e) 2-Bromopropionic acid, Pentadecyl ester

4. Conclusion

GC-MS has investigated five phytocompounds, two in ethanolic extract and three in aqua-ethanolic extract of roots of *U. picta*. Three compounds have been found to be reported as flavor, fragrance and food additive agents while one compound was found as antibacterial agent. Secondary metabolites produced by this plant may be of great interest for the pharmaceutical industry and medicinal chemistry research.

5. Acknowledgements

Authors pay their extreme gratitude to the Principal of Govt. Science College, Jabalpur and Director of Tropical Forest Research Institute, Jabalpur for extending help and support.

6. References

1. Julsing KM, Quax JW, Oliver K. The Engineering of Medicinal Plants: Prospects and Limitations of Medicinal Plant Biotechnology. ed. Medicinal Plant Biotechnology, 2007.
2. Ignacimuthu S, Sankarasivaraman K, Kesavan S. Medicoethno botanical survey among Kanikar tribals of mundentherai sanitary. Fitoterapia. 1998; 69:409-14.
3. Natarajan B, Paulsen BS, Pushpangadan P. An Ethnopharmacological study from the Coimbatore district, Tamil nadu, India: Traditional knowledge compared with Modern Biological Science. Pharmaceutical biology. 1999; 37:378-90.
4. Rajan S, Sethuraman M, Mukherjee PK. Ethnobiology of the Niligries Hills, India, Phytotherapy Research. 2002; 16:98-116.
5. Ayyanar M, Iganacimuthu S. Traditional knowledge of kani tribals in Kouthalai of Tirunelveli hills. Tamil Nadu, India. Journal of ethnopharmacology. 2005; 102:246-55.
6. Sandhy B, Thomas S, Isabel W, Shenbagavathai R. Ethnomedicinal plants used by the valaiyan community of Piranmalai hills (Reserved forest), Tamil Nadu, India. A pilot study. African Journal of Traditional, Complementary and Alternative Medicines. 2006; 3:101-14.
7. Saxena HO, Mohan B, Kakkar A. Assessment of variation in rhoifolin content in aerial parts of *Uraria picta* Desv. from different locations of Madhya Pradesh. Journal of Pharmacy Research. 2016; 10(5):185-190.
8. Joshi DD, Uniyal RC. Different chemo types of Gokhru (*Tribulus terrestris*): A herb used for improving physique and physical performance. International Journal of Green Pharmacy. 2008; 2:15861.
9. Vuorela P, Leinonen M, Saikku P, Tammela P, Rauha JP, Wennberg T *et al*. Natural products in the process of finding new drug candidates. Current Medicinal Chemistry. 2004; 11(11):1375-1389.
10. Bai S, Seasotiya L, Malik A, Bharti P, Dalal S. GC-MS analysis of chloroform extract of *Acacia nilotica* L. leaves. Journal of Pharmacognosy and Phytochemistry. 2014; 2(6):79-82.

11. Arora S, Kumar G. Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae). The Pharma Innovation Journal. 2017; 6(11):635-640.
12. McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold D *et al*. International Code of Botanical Nomenclature (Vienna Code). Koeltz Scientific Books, Konigstein – Germany, 2006.
13. Ohashi H, Iokawa Y. A Revision of *Uraria* (Leguminosae) in Taiwan. Taiwania. 2007: 52:177-183.
14. Yadav AK, Yadav D, Shanker K, Verma RK, Saxena AK, Gupta MM. Flavone glycoside based validated RP-LC method for quality evaluation of Prishniparni (*Uraria picta*). Chromatographia. 2009; 69(7, 8):653-658.
15. Pathak JM, Krishnamurthy R, Chandorkar MS, Gulkari V, Gupta R. Identification of high yielding genotypes of Dashmool Shalparni (*Desmodium gangeticum*) drug plant and its cultivation under high density planting. Indian Journal of Horticulture. 2005; 62(4):378-384.
16. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol 2. International Book Publishers Dehradun, India, 1993.
17. Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal plants of Bangladesh. BCSIR Laboratories, Chittagong, Bangladesh, 1994.
18. Anonymous. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization, Geneva, 2003.
19. Varghese S, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb.) seed. Journal of Acute Disease. 2013; 2(2):122-126002E.
20. Anonymous. [http://www.perflavory.com/docs/doc1450111.html]. [Visited on 18 July, 2020].
21. Anonymous. [https://pubchem.ncbi.nlm.nih.gov/compound/9-Octadecenoic-acid-_9Z_-_-hexyl-ester]. [Visited on 18 July, 2020].
22. Anonymous. [http://www.perflavory.com/upcn-p.htm]. [Visited on 18 July, 2020].
23. Kumar V, Bhatnagar AK, Srivastava JN. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. Journal of Medicinal Plants Research. 2001; 5(32):7043-7048.