Comparative analysis of major alkaloids in *Piper* species traded as 'Pippali' in South Indian markets: absence of the chief known constituent – piperine in selected samples

Vijayalakshmi Babu[1,*], Sandhya Kumari Singh[1], Padma Venkatasubramanian[1] and Padma Raj Gajurel[2]

[1]School of Life Sciences, Institute of Transdisciplinary Health Sciences and Technology, Foundation for Revitalisation of Local Health Traditions (FRLHT), 74/2, Jarakabande Kaval Attur PO, Yelahanka, Bengaluru 560 064, India
[2]Department of Forestry, North Eastern Regional Institute of Science and Technology (NERIST), Nirjuli 791 109, India

The major alkaloids in piper species traded in South Indian markets as the Ayurvedic drug, Pippali, have been studied using a rapid HPLC-based method. *Piper longum* is the accepted botanical source of Pippali. Bengaluru and Chennai markets contained mixtures of closely related species, *Piper peepuloides* and *Piper sylvaticum*. Chemical analysis showed that these samples do not contain the alkaloid piperine present in *Piper longum* fruits. Market samples from Trissur were identified as *Piper longum* and piperine was detected as one of the major alkaloids. All the samples contained pellitorine, another alkaloid reported in most peppers of the genus *Piper*. The two types of Pippali can therefore be easily differentiated on the basis of their HPLC profiles.

Keywords: Alkaloids, ayurvedic drug, HPLC, Pippali, *Piper longum*, piperine.

PLANT-BASED therapies are a significant component of Ayurvedic medicines. In ancient times, the Ayurvedic physician was well-aware of the source and properties of the medicinal plants being used, as these were mostly obtained from the local ecosystem. Furthermore, the physicians themselves would process the plant(s), prepare and administer the medicine. Hence, the method of sensory analysis was perhaps sufficient to determine the authenticity of the raw and/or processed drug. Today, due to industrialization of Ayurvedic medicine, raw and processed drugs are mostly procured from the market, often without checking the authenticity and quality. This has led to highly variable efficacy and safety of the Ayurvedic drugs due to possible adulteration, substitution, genetic modifications, geographical variations and sometimes incorrect processing of the medicinal plant(s)[1].

The Ayurvedic medicinal plants and their traditional preparations basically contain therapeutically important chemical constituents which are responsible for the biological or pharmacological activity. Thus, comprehensive chemical analysis of the raw drug/formulations is necessary in addition to botanical identification, for the authentication and standardization of Ayurvedic drugs. Identification of the active and/or major chemical compounds together with detailed chemical fingerprints using relevant analytical techniques is imperative to sustain and improve the quality of the traditional Ayurvedic medicines[2].

Pippali is one of the most widely used medicinal plants in Ayurveda and is present in over 300 formulations[3]. The main parts of the plant used are the fruits and the roots. The fruits are primarily implicated in the management of various diseases and associated symptoms of the respiratory tract such as cough, asthma, bronchitis, dysponea and allergies[4]. The plant (fruit) is also recommended for use in rheumatism, diabetes, diseases of the spleen, fevers and as an appetizer[5,6]. *Piper longum* L. belonging to the genus *Piper* (family Piperaceae) is the known and accepted botanical source of this drug[7]. Pippali represents the fruiting spikes and the Pippalimula the roots of the species. In India, the species mostly grows in the wild in tropical and subtropical climates in north eastern region and southern India and also cultivated in different parts of the country. It is normally a creeping and low climbing shrub preferring partially shaded moist areas. The modern pharmacological activities ascribed to *P. longum* (Pippali) fruits include antimicrobial, antifungal, antiasthmatic, antioxidant, antinflammatory, immunomodulatory action and anticancer[8]. The phytochemical groups reported in the fruits include alkaloids (piperamides and alkamides), lignans, esters, volatile oils and polyphenols[9]. The alkaloids are reported to be the major bioactive components present in the plant with piperine (Figure 1) being identified as the main active secondary metabolite among the alkaloids[10]. Piperine, is also known to primarily contribute to the pungency of the *P. longum* fruits[11]. This compound has thus also been chosen for quality assessment and standardization purposes by *Ayurvedic Pharmacopeia* of India and *Indian Pharmacopeia*[7]. Market samples of Pippali are sometimes different *Piper* species or even a mixture of two or more

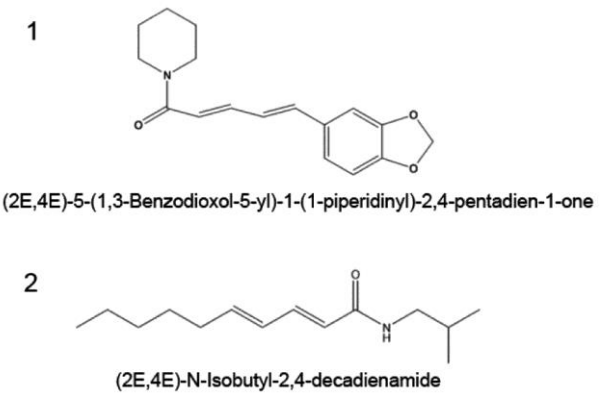

Figure 1. Structure of piperine (1) and pellitorine (2).

*For correspondence. (e-mail: vijayab@frlht.org)

Table 1. Accession details of Pippali samples

Location	Accession no.	Trade name	Month(s) and year of purchase/collection
FRLHT garden, Bengaluru*	P-B1	Pippali	October 2014–February 2015
K.R. market, Bengaluru	P-B2	Pippali	June 2014
K.R. market, Bengaluru	P-B3	Pippali	June 2015
Yelahanka Super market, Bengaluru	PN-B1	Black pepper	June 2015
Chennai market, Rasapachetty street	P-C1	Thippli	April 2015
Chennai market, Rasapachetty street	P-C2	Thippli	April 2015
Trissur market, Municipal bus stand	P-T1	Thippali	July 2015
Trissur market, Municipal bus stand	P-T2	Thippali	July 2015
Trissur market, Municipal bus stand	P-T3	Pippali	July 2015

*Fresh ripe fruits were collected and dried in an oven at 40°C for 48 h. All the market samples which were obtained as dried fruits were further dried in the oven for 6 h prior to storage.

species[12]. However, due to morphological similarities and without careful investigation, samples from other species are perhaps mistaken for *P. longum* L.

Here, we report the observed distinct trend in the content of the major alkaloid(s) in *Piper* species traded in South Indian markets as the Ayurvedic drug Pippali, using rapid high performance liquid chromatography (HPLC)-based identification and quantitation.

The Pippali (fruits) samples were procured from FRLHT garden, Bengaluru market, Chennai and Trissur markets (Table 1). The fruits collected from the FRLHT garden were authenticated taxonomically as *Piper longum* by a botanist. The market samples consisting of dried fruits were morphologically studied and authenticated. Among the various samples, the Trissur market samples were identified as purely *P. longum*. However, those procured from Chennai and Bengaluru markets were not that of *P. longum*. They were mostly the mixture of *P. peepuloides* Roxb. and *P. sylvaticum* Roxb.[13]. The dried fruiting spikes of *P. longum* appear very similar to that of *P. sylvaticum* and *P. peepulodes*, which are therefore often incorrectly identified as the former (for images see Supplementary Information online).

The major objective of this study was to compare the HPLC-based chemical profile and content of the major alkaloids in the different market samples of Pippali. A rapid extraction method using dichloromethane (DCM) as solvent was employed to decrease the total time required to prepare extracts when handling multiple samples[14]. *P. longum* fruits obtained from the FRLHT garden have been used as an authentic standard for this study. *P. nigrum* (from Bengaluru market) has been used as an additional control to check for reproducibility of the method. Preliminary screening of phytochemicals done by the method of Raman[15], indicated the presence of only alkaloids, fats and oils in all the DCM extracts. The HPLC profiles of DCM extracts of the different samples are shown in Figure 2. The peaks observed at 17.6 and 21.3 min were confirmed to be piperine (λ_{max} 340 nm, Figure 1) and pellitorine (λ_{max} 259 nm, Figure 1) respectively, by comparison of the retention time and UV spectra of the respective peaks with standards. The chromatograms were compared at three different wavelengths of 259, 275 and 340 nm. In all three samples from Trissur market (*P–T*), piperine and pellitorine were the major two alkaloids detected with the DCM extracts of P-T1, P-T2 and P-T3 showing similar profiles. There was over 90% match in the peaks detected. However, the pellitorine content was found to be much higher than the FRLHT garden sample. This is probably an effect of geographical variation. The HPLC chromatograms of Chennai (P-C1, P-C2) and Bengaluru (P-B1, P-B2) samples were similar but distinct differences could be observed when compared to the Trissur samples and the FRLHT garden, *P. longum*. Piperine was not detected at all within the detection limit of the HPLC method and pellitorine content was relatively higher in the Chennai and Bengaluru market samples. Soxhlet extraction, liquid nitrogen-based freeze–thaw method and maceration also did not yield any piperine from these samples (data not shown). The content of both alkaloids present in the various samples are shown in Table 2.

The absence of piperine in the Bengaluru and Chennai samples is an interesting result. This is because, piperine has been identified and well-studied as the major bioactive metabolite in *P. longum* (fruits), the accepted source of Pippali and is also known to be present in most piper species. Selective removal of piperine from powdered fruits of piper species has been reported[16]. However, complete removal from unpulverized fruits is highly improbable, as subjecting these to reflux/sonication/maceration with solvents for extended time periods, did not result in complete removal of piperine (data not shown). Hence, the variation in the alkaloid profile is probably an effect of genetic variability. Morphological examination has indicated that samples from these locations appear to be predominantly mixtures of fruits of *P. peepuloides* and *P. sylvaticum*. There are relatively fewer studies on these plants compared to *P. longum*. Piperine has been reported in stems and roots and pellitorine has been detected in the crushed seeds of *P. sylvaticum*[17]. In case of *P. peepuloides*, absence of piperine

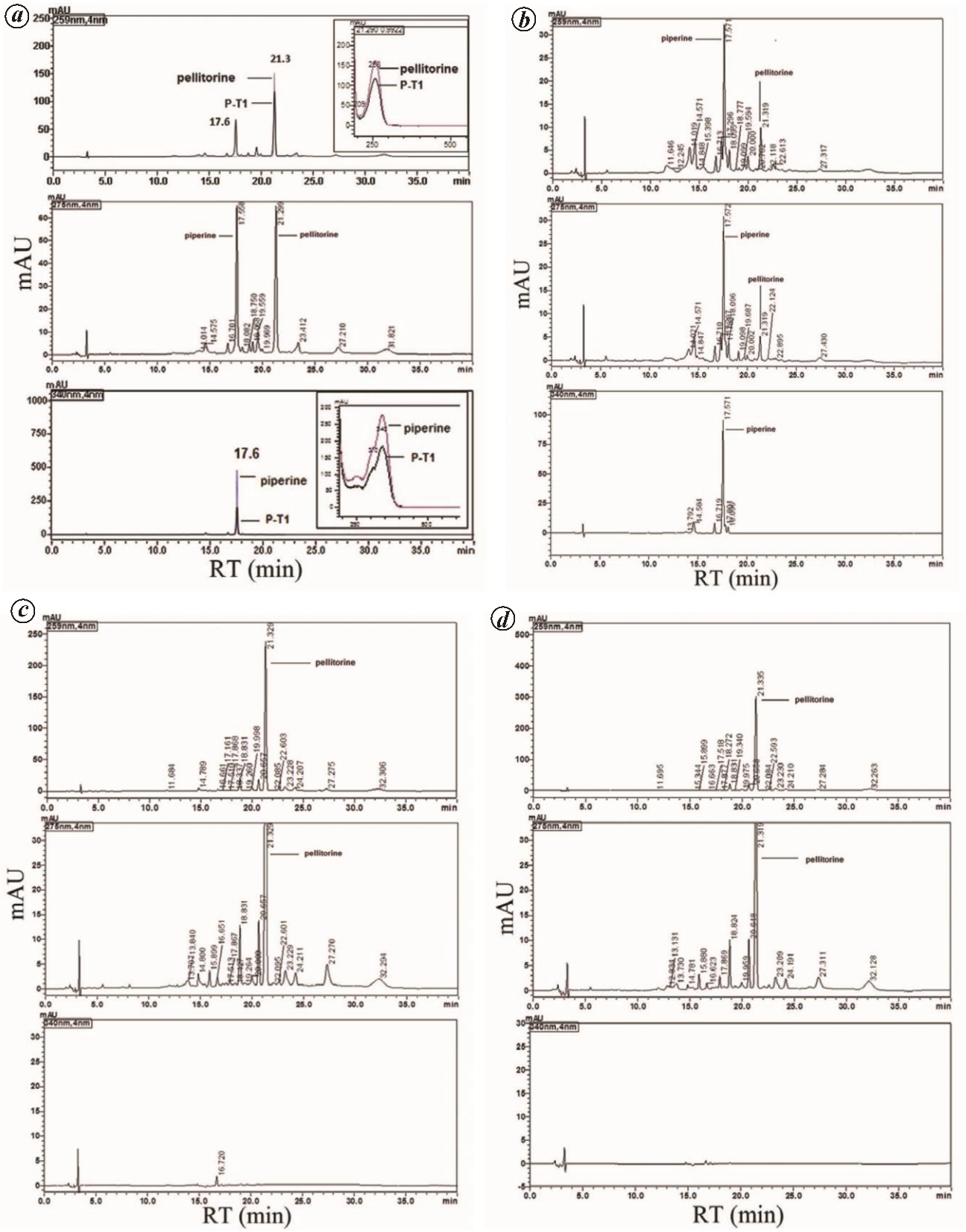

Figure 2. ***a***, High performance liquid chromatography (HPLC) profiles of dichloromethane (DCM) extract of Trissur market sample (P-T1) at 259 nm (inset – overlay of absorption spectra of pellitorine standard and P-T1 peak at 21.3 min), 275 nm and 340 nm (inset–overlay of absorption spectra of piperine standard and P-T1 peak at 17.6 min) respectively. ***b***, HPLC profiles of DCM extract of *Piper longum* FRLHT garden sample (P-B1 at 259, 275 and 340 nm respectively. ***c***, HPLC profiles of DCM extract of Bengaluru market sample (P-B2) at 259, 275 and 340 nm respectively. ***d***, HPLC profiles of DCM extract of Chennai sample (P-C1) at 259, 275 and 340 nm respectively.

Table 2. Piperine and pellitorine content in dichloromethane extract of the different samples – 1 h reflux method

Accession no.	Piperine content (w/w%, dry wt basis)*	Pellitorine content (w/w%, dry wt basis)*
PN-B1	2.59 ± 0.1	0.09 ± 0.004
P-B1	0.47 ± 0.03	0.08 ± 0.01
P-B2	–	1.53 ± 0.07
P-B3	–	1.61 ± 0.24
P-C1	–	1.69 ± 0.04
P-C2	–	1.27 ± 0.27
P-T1	0.82 ± 0.03	0.66 ± 0.003
P-T2	0.75 ± 0.01	0.65 ± 0.1
P-T3	0.75 ± 0.09	0.59 ± 0.05

*n = 3.

and isolation of pellitorine have been earlier reported, but the part of the plant used has not been specified[18]. These observations suggest that *P. longum* is absent in the Chennai and Bengaluru market samples of Pippali. Also, the fruits of *P. peepuloides* and *P. sylvaticum* probably lack piperine but contain pellitorine as the chief alkaloid. Further chemical and biological studies will reveal whether *P. peepuloides* and *P. sylvaticum* are adulterants or legitimate substitutes of *P. longum*.

The Ayurvedic drug Pippali has been botanically correlated to the plant *P. longum* L. In Bengaluru and Chennai markets, mixtures of *Piper* species closely related to *P. longum* are being sold as Pippali. HPLC-based chemical analysis has shown that these samples do not contain piperine, the chief bioactive phyto constituent of *P. longum*. Pellitorine, another alkaloid reported to be present in many peppers of the genus *Piper* was detected in all the samples. Therefore, these two market varieties of Pippali can be easily distinguished on the basis of their alkaloid profiles. This study highlights the importance of chemical profiling/analysis of medicinal plant sources along with botanical and DNA fingerprinting for authentication and to prevent adulteration. This study also iterates the immense and continuous need for scientific evaluation and documentation of market samples of plant-based raw drugs and their formulations.

1. Kumar, N., Dwivedi, K. N. and Ram, B., Adulteration and substitution of medicinal plant: a burning problem in herbal industry. *Int. J. Pharm. Biol. Sci. Arch.*, 2014, **5**(3), 13–18; Vasudevan, N. K., Yoganarasimhan, K. R., Kehava Murthy and Shantha, T. R., Studies on some south Indian market samples of Ayurvedic drugs II. *Anc. Sci. Life*, 1983, **3**(2), 60–66.
2. Cordell, G., Phytochemistry and traditional medicine – the revolution continues. *Phytochem. Lett.*, 2014, **10**, xxviii–xl; Rasheed, N. M., Nagaiah, K., Goud, P. R. and Sharma, V. U. M., Chemical marker compounds and their essential role in quality control of herbal medicines. *Ann. Phytomed.*, 2012, **1**(1), 1–8.
3. Anon., Ayurvedic Formulary of India, Department of Indian Systems of Medicine, Ministry of Health and Family Welfare Government of India, Part 1, 2000, 1st edn, p. 322; *FRLHT's Clinically Important Plants of Ayurveda*, CD, Version 1.0 Foundation for Revitalisation of Local Health Traditions, Bangalore, 2002.
4. Sharma, P. V., (editor-translator), *Caraka Samhita*, Chowkhambha Orientalia, New Delhi, 2005, vol. 2, pp. 23–24; Murthy, K. R. S. (translator). *Astanga Hridayam*, Krishna Das Academy, Varanasi, vol. 3, p. 397.
5. Pandey, G. S. (ed.), *Bhavaprakasa Nighantu*, Chowkhambha Bharati Academy, Varanasi, 2004, p. 15.
6. Tripathi, I. (ed.), *Rajanighantu*, Chowkhamba Krishna Das Academy, Varanasi, 2006, p. 134; Sharma, P. V., *Dhanvantari Nighantu*, Chowkhamba Orientalia, Varanasi, 2005, p. 83.
7. Agarwal, A. and Murali, B., *Quality Assessment of Selected Indian Medicinal Plants*, National Medicinal Plant Board, New Delhi and Natural Remedies Pvt Ltd, Bengaluru, 2010, vol. 1, pp. 181–207; *Indian Pharmacopeia*, Indian Pharmacopeia Commission, Ghaziabad, 2007, pp. 2057–2058; *Quality Standards of Indian Medicinal Plants*, ICMR, New Delhi, 2003, vol. 1, p. 168.
8. Zaveri, M., Khandar, A., Patel, S. and Patel, A., Chemistry and pharmacology of *Piper longum* L., *Int. J. Pharm. Sci. Rev. Res.*, 2010, **5**(1), 67–76.
9. Parmar, V. S. *et al.*, Phytochemistry of the genus *Piper*. *Phytochemistry*., 1997, **46**(4), 597–673.
10. Vasavirama, K. and Upender, M., Piperine. A valuable alkaloid from piper species. *Int. J. Pharm. Sci.*, 2014, **6**(4), 34–38.
11. Govindrajan, V. S. and Stahl, H. W., *Crit. Rev. Food Sci. Nutr.*, 1977, **9**(2), 115–225.
12. Dubey, R. B. and Sonawat, B. S., Current scenario of adulterants and substitutes of medicinal plants. A review. *J. Pharm. Sci. Innov*., 2015, **4**, 5; Yoganarasmhan, S. N., Nair, K. V., Shanta, T. R., Sudha, R. and Holla, B. V., Evaluation of South Indian market samples of Pippali. *BMEBR*, 1997, **XVIII**(1–2), 43–50; *The Wealth of India*, CSIR, New Delhi, 1969, vol. III, pp. 115–118.
13. Gajurel, P. R., Rethy, P., Kumar, Y. and Singh, B., *Piper species (Piperaceae) of North-East India (Arunachal Pradesh)*, Bishen Singh Mahendra Pal Singh, 2008, pp. 55–103.
14. Epstein, W. W., Netz, D. F. and Seidel, J. L., Isolation of piperine from black pepper. *J. Chem. Educ.*, 1993, **70**(7), 598–599.
15. Raman, N., *Phytochemical Techniques*, New India Publishing Agency, 2006, p. 19.
16. Raman, G. and Gaikar, V. G., Extraction of Piperine from *Piper nigrum* (Black Pepper) by hydrotropic solubilization. *Ind. Eng. Chem. Res.*, 2002, **41**(12), 2966–2976.
17. Banerji, J. and Dhara, K. P., Lignan and amides from *Piper sylvaticum*. *Phytochemistry*, 1974, **13**(10), 2327–2328; Mahanta, P. K., Ghanim, A. and Gopinath, K. W., Chemical constituents of *Piper sylvaticum* (Roxb) and piper boehmerifolium (Wall). *J. Pharm. Sci.*, 1974, **63**(7), 1160–1161; Banerji, A., Rej, R. N. and Ghosh, P., Isolation of N-isobutyldeca-trans-2-trans-4-dienamide from *Piper sylvaticum*. *Experientia*, 1974, **30**(3), 223–224.
18. Dhar, K. L. and Atal, C. K., *Indian J. Chem.*, 1967, **5**, 588–589.

ACKNOWLEDEGMENTS. V.B. and S.K.S. thank Mr Darshan Shankar, Institute of Transdisciplinary Health Sciences and Technology, FRLHT, Bengaluru for support from the Darshan Shankar Endowment Fund. V.B. also thanks DST for Young Scientist award. We also thank Dr Noorunnisa Begum for authentication of samples. Prof. D. K. Ved, Prof. M. N. B. Nair and Prof. K. Yoganarasimhan are thanked for suggestions. SIF facility, IISc and CCAMP, NCBS-TIFR are thanked for the NMR and HRMS data respectively.

Received 21 December 2015; revised accepted 10 June 2016

doi: 10.18520/cs/v111/i9/1507-1510