In summary, the red mud samples of NALCO contain about 77% of very fine particles, i.e. below 45 μm, having TREE of 433 ppm. Monazite and zircon are the main REE-mineral phases present as discrete grains along with other minerals such as gibbsite, hematite, goethite, ilmenite, rutile and magnetite. These REE-mineral phases may be concentrated using suitable mineral processing techniques such as floatation and magnetic separation. In view of the current demand and geo-political scenario, there is a need to secure critical resources like REE. Red mud could be a viable feedstock for extracting REE, provided there is a complete understanding of its REE characteristics. This makes the huge accumulation of red mud a resource rather than a waste product. As mineralogy plays a vital role in mineral beneficiation or any route of metal extraction, this study may help in the recovery of REE from Indian red mud in the future.

1. Fois, A., Lallai, G. and Mura, Sulfur dioxide absorption in a bubbling reactor with suspensions of Bayer red mud. *Ind. Eng. Chem. Res.*, 2007, **46**, 6770–6776.
2. Mohapatra, B. K., Rao, M. B. S., Rao, R. B. and Paul, A. K., Characteristics of red mud generated at NALCO Refinery, Damanjodi, India. In Proceedings of 129th the Minerals, Metals and Materials Society, Annual Meeting (eds Eckert, C. E. and Peterson, R. D.), Nashville, Tennessee, USA, 2000, pp. 161–165.
3. Abhilash, Sinha, S., Sinha, M. K. and Pandey, B. D., Extraction of lanthanum and cerium from Indian red mud. *Int. J. Min. Proc*., 2014, **127**, 70–73.
4. Glenister, D. J. and Thornber, M. R., Alkalinity of red mud and its applications for management of acid wastes. *Chemica*, 1985, **85**, 100–113.
5. Hind, A. R., Bhargava, S. K. and Grocott, S. C., The surface chemistry of Bayer process solids: a review. *Colloid. Surf. A*, 1999, **146**(1–3), 359–374.
6. Liu, X. and Zhang, N., Utilization of red mud in cement production: a review. *Waste Manage. Res*., 2011, **29**, 1053–1063.
7. Agrawal, A., Sahu, K. K. and Pandey, B. D., Solid waste management in nonferrous industries in India. *Resour. Conserv. Recycl*., 2004, 99–120.
8. Abhilash, Sinha, S., Meshram, P. and Pandey, B. D., Red mud: a secondary resource for rare earth elements. In Proceedings of International Bauxite, Alumina and Aluminium Symposium, Visakhapatnam, India, 2014, vol. 3, pp. 148–162.
9. Power, G., Grafe, M. and Klauber, C., Bauxite residue issues: I. Current management, disposal and storage practices. *Hydrometallurgy*, 2011, **108**, 33–45.
10. Akcil, A., Akhmadiyeva, N., Abdulvaliyev, R., Abhilash and Meshram, P., Overview on extraction and separation of rare earth elements from red mud: focus on scandium. *Miner. Process Extract. Metall. Rev.*, 2018, **39**(3), 145–151.
11. Kovács, T., Horváth, M., Csordás, A., Bátor, G. and Tóth-Bodrogi, E., Tobacco plant as possible biomonitoring tool of red mud dust fallout and increased natural radioactivity. *Heliyon*, 2020, **6**, e03455.
12. Alam, S., Das, B. K. and Das, S. K., Dispersion and sedimentation characteristics of red mud. *J. Hazard. Toxic Radioact. Waste*, 2018, **22**, 04018025.
13. Liu, Z. and Li, H., Metallurgical process for valuable elements recovery from red mud – a review. *Hydrometallurgy*, 2015, **155**, 29–43.
14. Sun, Y. F., Dong, F. Z. and Liu, J. T., Technology for recovering iron from red mud by Bayer process (in Chinese). *Met. Mine*, 2009, **9**, 176–178.
15. Rivera, R. M., Xakalashe, B., Ounoughene, G., Binnemans, K., Friedrich, B. and Van Gerven, T., Selective rare earth element extraction using high-pressure acid leaching of slags arising from the smelting of bauxite residue. *Hydrometallurgy*, 2009, **184**, 162–174.
16. Chaikin, L., Shoppert, A., Valeev, D., Loginova, I. and Napol Skikh, J., Concentration of rare earth elements (Sc, Y, La, Ce, Nd, Sm) in bauxite residue (red mud) obtained by water and alkali leaching of bauxite sintering dust. *Minerals*, 2020, **10**(500), 1–13.
17. Binnemans, K., Jones, P. T., Blanpain, B., Gerven, T. V., Yang Y., Walton, A. and Buchert, M., Recycling of rare earths: critical review. *J. Clean. Prod*., 2013, **51**, 1–22.
18. Deady, E., Mouchos, E., Goodenough, K., Williamson, B. and Wall, F., Rare earth elements in Karst-bauxites: a novel untapped European resource. In Proceedings of the 1st Europe Rare Earth Resources Conference, Milos Island, Greece, 2014, pp. 397–409.
19. Sinha, S., Sinha, M. K. and Pandey, B. D., Extraction of lanthanum and cerium from Indian red mud. *Int. J. Miner. Process*., 2014, **127**, 70–73.
20. Nath, H. and Sahoo, A., A study on the characterization of red mud. *Int. J. Appl. Bioeng*., 2014, **8**(1), 1–4.

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Chemical constituents of essential oil from leaves of an invasive weed *Ageratina adenophora* in Central Nepal

Khadka Bahadur Pal[1], Seeta Pathak[2], Tayer Mohamad Miya[3], Tej Bahadur Darji[2,4], Gunanand Pant[5], Ramesh Raj Pant[6], Lok Ranjan Bhatt[7] and Lal B. Thapa[2,*]

[1]Department of Chemistry, Trichandra Campus, Tribhuvan University, Kathmandu, Nepal
[2]Central Department of Botany, Institute of Science and Technology, Tribhuvan University, Nepal
[3]Department of Food Technology and Quality Control, Government of Nepal, Kathmandu, Nepal
[4]Department of Forest Resources, Ministry of Forest and Environment, Plant Research Center, Jumla, Nepal
[5]Department of Biology, Kailali Multiple Campus, Far Western University, Kailali, Nepal
[6]Central Department of Environmental Science, Institute of Science and Technology, Tribhuvan University, Nepal
[7]Biological Resource Unit, Nepal Academy of Science and Technology, Lalitpur, Nepal

This study aims to identify volatile chemical components in the essential oil of an invasive plant, *Ageratina adenophora*, from Central Nepal. Leaf samples of *A. adenophora* were collected, and the chemical composition

*For correspondence. (e-mail: lal.thapa@cdb.tu.edu.np)

of essential oil was analysed by gas chromatography (GC) and GC–mass spectrometry (GC/MS). A total of 27 components in the oil were identified. The major compounds were cadinol, bisabolol and bornyl acetate. The amount of valencene, elemol and 2(1H)-naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar(4a.alpha.,6.alpha.,8a.beta.]- varied with samples collected from different elevations. Five compounds, viz. cloven, β-gurjuene, β-cubebene, verbenone and cis-β-farnesene were rarely reported previously from *A. adenophora*. Being an invasive and medicinal plant, *A. adenophora* is found in varied ecological conditions. Further studies on the variation in amount and composition, phytotoxicity and benefits of such compounds are recommended.

Keywords: *Ageratina adenophora*, chemical constituents, essential oil, gas chromatography, mass spectrometry.

AGERATINA adenophora (Spreng.) R.M. King and H. Rob. (Crofton weed) is an invasive alien species naturalized in several countries in Asia, Australia, Africa and Europe[1,2]. It is a perennial herbaceous plant belonging to the family Asteraceae having an erect glandular stem, dark green leaves and white flowers[1,3]. It has invaded roadsides, fallow lands, disturbed forests and surroundings of agricultural fields[4,5]. Small, numerous and light seeds which are spread by wind and the vegetative means of reproduction and the ability to produce several allelochemicals are the invasive characteristics of this species[6–8].

In the invaded range *A. adenophora* has changed the composition and diversity of the native vegetation[9,10], altered the soil microbial communities[11,12] and soil nutrients[13]. These changes are caused by the allelochemicals released from *A. adenophora*[14,15]. An easy way to release allelochemicals from aerial parts of the plant to the soil is rainwater[16]. In addition, *A. adenophora* releases a unique odour (volatiles) from the glandular stem and leaves, having several organic compounds, which are also supposed to have a negative impact on native vegetation[17,18].

A. adenophora has been naturalized in Nepal and is distributed throughout the country[19] invading several terrestrial ecosystems with negative impacts on the native vegetation[3,17,20]. As Nepal has diverse climatic and geographical variations, a variety of allelochemicals might have been released by *A. adenophora* in different locations. Identification of such allelochemicals will have significance to understand the plant invasiveness and its potential impacts on the native ecosystems. On the other hand, controlling and managing naturalized *A. adenophora* is challenging issue. One method of control and management could be utilization of the plant by recognizing its various beneficial facets. Various compounds in the plant have several pharmacological activities, including antimicrobial and larvicidal[21–23]. Hence, there is an option of utilizing this plant to produce useful phytochemicals for the benefit of human beings. Therefore, screening of compounds should be given the first and high priority. The literature survey revealed that the essential oil of *A. adenophora* contains a variety of volatile chemicals and other biologically active constituents[24–27]. However, information from Nepal (the Himalayan region) is meagre. This prompted us to carry out a chemical analysis of *A. adenophora* collected from proximate locations differing by about 100 m elevation in Kathmandu Valley, Bagmati Province, Nepal.

Fresh leaves of *A. adenophora* were collected (1500 g) from the Champadevi hill forest in the southwestern part of Kirtipur Municipality, Kathmandu, Nepal, in January 2019. They were collected from two *A. adenophora* invaded sites (site A, 1504 m amsl, 27°39′420″N/085°15′019″E and site B, 1632 m amsl, 27°39′311″N/085°14′878″E) in the forest. A total of six leaf samples were collected, three from each site. They were tagged as SA (sample A, sub-samples = A1, A2 and A3) for the samples from site A and SB (sample B, sub-samples = B1, B2 and B3) for those from site B. The samples were stored in a refrigerator (4°C) until further use.

Essential oil from each sub-samples was isolated separately using Clevenger apparatus (300 ml) at 80°C by the process of hydrodistillation (3 h). The amount of leaf used for hydro-distillation was 150 g with 1.5 l water. The oil was collected and transferred to a clean sealed glass vial and stored at 0°–4°C until further analyses.

The Shimadzu QP2010 Plus operating system in electron impact (EI) mode was used for gas chromatography–mass spectrometry (GC–MS) analysis. Essential oil extracted was subjected to GC fitted with Rtx-5MS column (30 m × 0.25 mm id, film thickness of 0.25 μm), coupled to a QP2010 Plus mass detector. Column oven temperature was maintained at 80°C for 2 min and then gradually increased to 280°C at the rate of 6°C/min, which was finally held isothermally for 5 min. Injector temperature was adjusted to 220°C. Helium was the carrier gas with a total flow rate 18.5 ml/min. Volume of the manually injected oil sample (diluted by 1/100 in ethanol, v/v) was 1 μm (split mode, split ratio 1 : 15) with 1.03 ml/min through the column. An electron ionization system with ionization energy of 70 eV was used on an Rtx-5MS mass spectrometer connected to GC–MS solution software. Spectra were obtained over the mass range 45–500 m/z at a scan speed of 1000 scan/ms. The chromatogram was analysed, and each peak was checked by determining the per cent area on the chromatogram, retention time, spectrum, and base peak in the National Institute of Standards and Technology (NIST05s) library. The percentage relative abundances was calculated as follows

$$\text{Relative abundance (\%)} = \frac{\text{Peak area of the component}}{\text{Total sum of all peak areas}} \times 100.$$

The independent sample *t*-test was used to compare the difference in relative abundance (%) of the compounds present

Table 1. Compounds identified in sample A (SA) and sample B (SB) showing percentage of relative abundance (RA), *R*-time and *R*-index

Compound	SA		SB			
	% RA	*R*-time	% RA	*R*-time	*R*-index	*R*-index (from the literature)
2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxyl-methyl)ethenyl]-4,8a-dimethyl, [4ar-(4a.alpha.,6.alpha.,8]	1.28**b**	24.29	1.73**a**	24.29	1888	–
2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)	7.12	19.17	7.72	19.16	1438	–
Bisabolol[i]	12.73	18.89	13.31	18.88	1625	1682.8[a]
Borneol[ii]	1.20	8.10	0.96	8.10	1138	1166.2[a]
Bornyl acetate[ii]	11.85	10.72	11.44	10.72	1277	1283.5[a]
Cadinol[i]	18.45	17.99	19.17	17.97	1580	1651.9[a]
Caryophyllene oxide[i]	1.49	16.97	1.50	16.97	1507	1580.6[a]
cis-β-Farnesene[i]	AB		0.88	14.25	1440	1414.5[a]
Clovene[i]	3.30	19.64	AB		1446	1465[b]
Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)	6.78	21.01	4.54	20.97	1296	–
Elemol[i]	6.14**a**	16.27	4.97**b**	16.27	1522	1547.5[a]
Spathulenol[i]	3.70	18.35	3.97	18.34	1530	1575[c]
Farnesyl acetate[i]	1.41	16.80	2.37	17.49	1834	1919.5[a]
Geranylgeranyl acetate[iii]	AB		1.57	16.80	2316	1379.9[a]
Nerolidol[i]	1.87	16.80	1.68	16.45	1564	1560.9[a]
Retinol[iii]	2.94	19.63	2.79	19.65	2238	2453.0[d]
Valencene[i]	1.12	22.24	AB		1474	1491.7[a]
Verbenone[ii]	AB		4.60	20.99	1119	1206.2[a]
Viridiflorol[i]	AB		2.38	21.85	1536	1590.8[a]
α-Bergamotene[i]	AB		0.75	13.87	1430	1414.5[a]
α-Cedrene[i]	2.08	23.34	2.04	23.34	1403	1412.2[a]
β-Bisabolene[i]	AB		1.14	15.36	1500	1508.4[a]
β-Cubebene[i]	AB		4.64	14.90	1339	1386.6[a]
β-Gurjurene[i]	AB		17.24	18.00	1403	1431.2[a]
β-Thujene[ii]	AB		1.03	15.82	879	971.0[e]
γ-Elemene[i]	AB		0.87	15.20	1431	1436.4[a]
γ-Muurolene[i]	3.59	14.89	4.62	14.90	1435	1476.2[a]

Note: AB, Absent. The letters 'a' and 'b' in the average % RA represent significant difference (independent sample *t*-test, $P < 0.05$). *R*-index values: [a]Babushok *et al.*[39] (dimethylsilicone with 5% phenyl groups); [b]Hedin *et al.*[40]; [c]Tellez *et al.*[41], [d]Furr *et al.*[42], [e]Weyerstahl *et al.*[43]. Chemical classes: [i]Sesquiterpene, [ii]monoterpene and [iii]diterpene.

in samples A and B. The compounds present in the three subsamples of both samples A and B were used in the analysis.

A total of 27 major and minor components were identified from both leaf samples. Table 1 lists the compounds identified in SA and SB with their area percentage. A total of 15 compounds were common in both the samples collected. Major compounds in both the samples were cadinol (SA = 18.45%; SB = 19.17%), bisabolol (SA = 12.73% and SB = 13.31%) and bornyl acetate (SA = 11.85% and SB = 11.44%) (Table 1).

Other common compounds in both the samples were 2H-cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl) (SA = 7.12% and SB =7.72%); cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl) (SA = 6.78% and SB = 4.54%); elemol (SA = 6.14% and SB = 4.97%); γ-muurolene (SA = 3.59% and SB = 4.62%); espathulenol (SA = 3.70% and SB = 3.97%) and retinol (SA = 2.94% and SB = 2.79%) (Table 1). The minor compounds were 2(1H)-naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl, [4ar-(4a.alpha.,6.alpha.,8a.beta.], borneol, caryophyllene oxide, farnesyl acetate, nerolidol and α-cedrene.

Independent sample *t*-test showed no differences in the amount of 2H-cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl), bisabolol, bornyl acetate, caryophyllene oxide, nerolidol and α-cedrene between samples A and B. However, the amount of 2(1H)-naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl, [4ar-(4a.alpha.,6.alpha.,8a.beta.] was significantly high in sample B (df = 4, t = –3.38, P = 0.028), whereas the amount of elemol was high in sample A (df = 4, t = 5.266, P = 0.006) (Table 1).

Two of the compounds, cloven (3.30%) and valencene (1.12%) were found only in sample A (Table 1). Cloven was found only in samples A1 and A2, and valencene only in sample A1. The other ten compounds were found only in sample B (Table 1). The major compound in sample B was β-gurjurene (17.24%), followed by β-cubebene (4.64%), verbenone (4.60%), viridiflorol (2.38%) and β-bisabolene (1.14%). α-Bergamotene, γ-elemene and cis-β-farnesene were the trace compounds (<0.1%) (Table 1).

Majority of the compounds identified from both the samples, such as bisabolol, borneol, bornyl acetate, cadinol, caryophyllene oxide, elemol, espathulenol, nerolidol and

α-cedrene are the most reported constituents in the essential oil of *A. adenophora* leaves from different parts of the world[14,24,28]. However, compounds such as farnesyl acetate and retinol in our samples have not been reported often previously. Similarly, 2(1H)-naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl, [4ar-(4a.alpha.,6.alpha.,8a.beta.] and 2H-cyclopropa[g]-benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl) are also rarely reported by previous studies. Giri *et al.*[29] have reviewed the phytochemicals from *A. adenophora* and listed more than 95 compounds, but the aforementioned compounds from our analysis have not been mentioned.

Viridiflorol was reported by Pala-Paul *et al.*[25] in *A. adenophora.* Similarly, β-bisabolene was reported by Pala-Paul *et al.*[25], and Subba and Kandel[26]. Pandey *et al.*[30] have found α-bergamotene and γ-elemene in *A. adenophora* showing antibacterial activity. Compounds such as cloven, valencene, β-gurjuene, β-cubebene, verbenone and cis-β-farnesene are rarely reported in *A. adenophora.* Our results indicate that the quantity of certain compounds in the volatile oil varies within even a short range of elevation. Except for cloven and valencene, the other compounds were found in the leaves from higher elevation (1632 m amsl). According to Talebi *et al.*[31], the elevation gradient has a greater impact on essential oil composition and the amount of certain monoterpenes decreases with elevation while the amount of oxygenated compounds increases. Several factors such as light, temperature and moisture change with elevation and affect the quantity and composition of essential oil in plants[32].

Several studies have proved the allelopathic effects of *A. adenophora* on native species[17], crops[33] and soil microbes[34]. The allelochemicals such as α-phellandrene, camphene, ρ-cymene, 2-carene, α-pinene, limonene and (z)-3-hexen-1-ol present in the aerial and underground parts of *A. adenophora* are phytotoxic[35]. In addition, β-ocimene, linalool, α-farnesene, β-farnesene are released in response to herbivory in plants[36]. Such anti-herbivory chemicals in *A. adenophora* might have increased its adaptiveness to a wide range of environments[36–38].

As Nepal has diverse climatic and geographical variations, a variety of allelochemicals might be released by *A. adenophora* in different locations. Identifying such allelochemicals would have significance to understanding the plant invasiveness and its potential impacts on the native ecosystems. On the other hand, control and management of naturalized *A. adenophora* has become a challenge. It is often suggested that the utilization of such plants by recognizing their beneficial facets could be one way to control them. In this regard, *A. adenophora* can isolate antimicrobial, larvicidal and medicinal compounds[21–23]. The compounds having such properties have been identified as major compounds in this study (Table 1).

In conclusion, the present study identified 27 compounds from the essential oil of *A. adenophora* in Kathmandu valley, Nepal. The major compounds were cadinol, bisabolol and bornyl acetate. The composition of chemical compounds and the amount of valencen, 2(1H)-naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxylmethyl)ethenyl]-4,8a-dimethyl, [4ar-(4a.alpha.,6.alpha.,8a.beta.] and elemol differed with sampling location. As *A. adenophora* is a highly problematic invasive plant found in varied ecological conditions, further studies on spatial variations in the amount and composition of phytochemicals are recommended.

Conflict of interest: The authors declare that there is no conflict of interest.

1. Muniappan, R., Raman, A. and Redd, G. V. (eds), *Ageratina adenophora* (Sprengel) King and Robinson (Asteraceae). In *Biological Control of Tropical Weeds using Arthropods*, Cambridge University Press, Cambridge, UK, 2009, pp. 1–16.
2. Yang, G. Q., Qiu, W. R., Jin, Y. N. and Wan, F. H., Potential allelochemicals from root exudates of invasive *Ageratina adenophora*. *Allelopathy J*., 2013, **32**, 233.
3. Tiwari, S., Siwakoti, M., Adhikari, B. and Subedi, K., An inventory and assessment of invasive alien plant species of Nepal. IUCN – The World Conservation Union, Nepal, 2005, p. 114.
4. Thapa, L. B., Thapa, H. and Magar, B. G., Perception, trends and impacts of climate change in Kailali District, Far West Nepal. *Int. J. Environ*., 2015, **4**, 62–76.
5. Thapa, L. B., Kaewchumnong, K., Sinkkonen, A. and Sridith, K., Plant communities and *Ageratina adenophora* invasion in lower montane vegetation, Central Nepal. *Int. J. Ecol. Dev.*, 2016, **31**, 35–49.
6. Tripathi, R. S., Yadav, A. S. and Kushwaha, S. P. S., Biology of *Chromolaena odorata*, *Ageratina adenophora* and *Ageratina riperia*: a review. In *Invasive Alien Plants: An Ecological Appraisal for the Indian Subcontinent* (eds Bhatt, J. R. *et al.*), CAB International, Cambridge, UK, 2012, pp. 3–56.
7. Zhao, X., Zheng, G. W., Niu, X. M., Li, W. Q., Wang, F. S. and Li, S. H., Terpenes from *Eupatorium adenophorum* and their allelopathic effects on *Arabidopsis* seeds germination. *J. Agric. Food Chem.*, 2009, **57**, 478–482.
8. Lallianrawna, S., Muthukumaran, R., Ralte, V., Gurusubramanian, G. and Kumar, N. S., Determination of total phenolic content, total flavonoid content and total antioxidant capacity of *Ageratina adenophora* (Spreng.) King & H. Rob. *Sci. Vis*., 2013, **13**, 149–156.
9. Tererai, F. and Wood, A. R., On the present and potential distribution of *Ageratina adenophora* (Asteraceae) in South Africa. *S. Afr. J. Bot*., 2014, **95**, 152–158.
10. Thapa, L. B., Kaewchumnong, K., Sinkkonen, A. and Sridith, K., 'Soaked in rainwater' effect of *Ageratina adenophora* on seedling growth and development of native tree species in Nepal. *Flora*, 2020, **263**, 151554.
11. Niu, H. B., Liu, W. X., Wan, F. H. and Liu, B., An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant Soil*, 2007, **294**, 73–85.
12. Xu, C. W. *et al.*, Changes in non-symbiotic nitrogen-fixing bacteria inhabiting rhizosphere soils of an invasive plant *Ageratina adenophora*. *Appl. Soil Ecol.*, 2012, **54**, 32–38.
13. Bajpai, D. and Inderjit, I., Impact of nitrogen availability and soil communities on biomass accumulation of an invasive species. *AoB Plants*, 2013, **5**, plt045.
14. Kundu, A., Saha, S., Ahluwalia, V. and Walia, S., Plant growth inhibitory terpenes from *Eupatorium adenophorum* leaves. *J. Appl. Bot. Food Qual*., 2013, **86**, 33–36.

15. Wan, H., Liu, W. and Wan, F., Allelopathic effect of *Ageratina adenophora* (Spreng.) leaf litter on four herbaceous plants in invaded regions. *J. Eco-Agric*., 2011, **19**, 130–134.
16. Thapa, L. B., Deuba, R., Oli, N., Singh, U. K. and Jha, S. K., Invasion status of alien weeds in the historical Chobhar area of Kathmandu valley, Nepal. *Indian J. Weed Sci*., 2020, **52**, 405–407.
17. Thapa, L. B., Kaewchumnong, K., Sinkkonen, A. and Sridith, K., Plant invasiveness and target plant density: high densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora*. *Weed Res.*, 2017, **57**, 72–80.
18. Thapa, L. B., Kaewchumnong, K., Sinkkonen, A. and Sridith, K., Airborne and belowground phytotoxicity of invasive *Ageratina adenophora* on native species in Nepal. *Plant Ecol.*, 2020, **221**, 883–892.
19. Shrestha, B. B., Invasive alien plant species in Nepal. In *Frontiers of Botany* (eds Jha, P. K., Siwakoti, M. and Rajbhandari, S.), Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, 2016, pp. 269–284.
20. Darji, T. B. *et al.*, Phytotoxic effects of invasive *Ageratina adenophora* on two native subtropical shrubs in Nepal. *Sci. Rep*., 2021, **11**, 1–9.
21. Torres-Barajas, L., Rojas-Vera, J., Morales-Mendez, A., Rojas-Fermin, L., Lucena, M. and Buitrago, A., Chemical composition and evaluation of antibacterial activity of essential oils of *Ageratina jahnii* and *Ageratina pichinchensis* collected in Mérida, Venezuela. *Biol. Latinoam. Caribe Plantas Med. Aromát*., 2013, **12**, 92–98.
22. Rajeswary, M., Govindarajan, M., Murugan, K., Hwang, J. S., Barnard, D. R., Amsath, A. and Muthukumaran, U., Ovicidal efficacy of *Ageratina adenophora* (family: Asteraceae) against *Anopheles stephensi* (Diptera: Culicidae). *Int. J. Pure Appl. Zool*., 2014, **2**, 196–199.
23. Zhang, M. *et al.*, Bioactive quinic acid derivatives from *Ageratina adenophora*. *Molecules*, 2013, **18**, 14096–14104.
24. Padalia, R. C., Bisht, D. S., Joshi, S. C. and Mathela, C. S., Chemical composition of the essential oil from *Eupatorium adenophorum* Spreng. *J. Essent. Oil Res.*, 2009, **21**, 522–524.
25. Pala-Paul, J., Perez-Alonso, M. J., Velasco-Negueruela, A. and Sanz, J., Analysis by gas chromatography–mass spectrometry of the volatile components of *Ageratina adenophora* Spreng., growing in the Canary Islands. *J. Chromatogr. A*, 2002, **947**, 327–331.
26. Subba, B. and Kandel, R. C., Chemical composition and bioactivity of essential oil of *Ageratina adenophora* from Bhaktapur district of Nepal. *J. Nepal Chem. Soc*., 2012, **30**, 78–86.
27. Samuel, L., Muthukumaran, R. B., Gurusubramanian, G. and Senthilkumar, N., Larvicidal activity of *Ipomoea cairica* (L.) Sweet and *Ageratina adenophora* (Spreng.) King & H. Rob. plant extracts against arboviral and filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Exp. Parasitol*., 2014, **141**, 112–121.
28. Weyerstahl, P., Marschall, H., Seelmann, I. and Kaul, V. K., Constituents of the flower essential oil of *Ageratina adenophora* (Spreng.) K. et R. from India. *Flavour. Fragr. J*., 1997, **12**, 387–396.
29. Giri, S., Sahu, R., Paul, P., Nandi, G. and Dua, T. K., An updated review on *Eupatorium adenophorum* Spreng. [*Ageratina adenophora* (Spreng.)]: traditional uses, phytochemistry, pharmacological activities and toxicity. *Pharmacol. Res.-Mod. Chin. Med.*, 2022, 100068.
30. Pandey, A. K., Mohan, M., Singh, P., Palni, U. T. and Tripathi, N. N., Chemical composition, antibacterial and antioxidant activity of essential oil of *Eupatorium adenophorum* Spreng. from eastern Uttar Pradesh, India. *Food Biosci*., 2014, **7**, 80–87.
31. Talebi, S. M., Nohooji, M. G., Yarmohammadi, M., Khani, M. and Matsyura, A., Effect of altitude on essential oil composition and on glandular trichome density in three *Nepeta* species (*N. sessilifolia*, *N. heliotropifolia* and *N. fissa*). *Mediterr. Bot.*, 2019, **40**, 81–93.
32. Mahdavi, M., Jouri, M. H., Mahmoudi, J., Rezazadeh, F. and Mahzooni-Kachapi, S. S., Investigating the altitude effect on the quantity and quality of the essential oil in *Tanacetum polycephalum* Sch.-Bip. polycephalum in the Baladeh region of Nour, Iran. *Chin. J. Nat. Med.*, 2013, **11**, 553–559.
33. Jiao, Y., Sang, Y., Yang, L., Wang, Y. Q., Wu, Y. K., Du, R. W. and Yuan, L., Effects of fresh and composted *Ageratina adenophora* on physiology of three Solanaceae vegetables and yield and quality of pepper. *Sci. Agric. Sin*., 2016, **49**, 874–884.
34. Balami, S., Thapa, L. B. and Jha, S. K., Effect of invasive *Ageratina adenophora* on species richness and composition of saprotrophic and pathogenic soil fungi. *Biotropia*, 2017, **24**, 212–219.
35. Zhang, F., Guo, J., Chen, F., Liu, W. and Wan, F., Identification of volatile compounds released by leaves of the invasive plant Crofton weed (*Ageratina adenophora*, Compositae), and their inhibition of rice seedling growth. *Weed Sci.*, 2012, **60**, 205–211.
36. Hare, J. D., Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annu. Rev. Entomol*., 2011, **56**, 161–180.
37. Balami, S. and Thapa, L. B., Herbivory damage in native *Alnus nepalensis* and invasive *Ageratina adenophora*. *Bot. Oriental.*, 2017, **11**, 7–11.
38. Paré, P. W. and Tumlinson, J. H., Plant volatiles as a defense against insect herbivores. *Plant Physiol*., 1999, **121**, 325–332.
39. Babushok, V. I., Linstrom, P. J. and Zenkevich, I. G., Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data*, 2011, **40**, 043101.
40. Hedin, P. A., McKibben, G. H. and Dollar, D. A., Role of ground trash volatiles in the selection of hibernation sites by boll weevils. *J. Agric. Food Chem.*, 2000, **48**, 3673–3676.
41. Tellez, M. R., Canel, C., Rimando, A. M. and Duke, S. O., Differential accumulation of isoprenoids in glanded and glandless *Artemisia annua* L. *Phytochemistry*, 1999, **52**, 1035–1040.
42. Furr, H. C., Zeng, S., Clifford, A. J. and Olson, J. A., Capillary gas chromatography of retinoids (vitamin A compounds) and apo-retinoids: determination of Kováts retention indices. *J. Chromatogr*., 1990, **527**, 406–413.
43. Weyerstahl, P., Marschall, H., Thefeld, K. and Rustaiyan, A., Constituents of the essential oil of *Tanacetum* (syn. Chrysanthemum) *fruticulosum* Ledeb. from Iran. *Flavour. Frag J*., 1999, **14**, 112–120.

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