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RESEARCH ARTICLES

Bioprospecting of ornamental and weed plants for hydroxynitrile lyase activity and characterization of novel hydroxynitrile lyases (HNLs) of *Euphorbia mili* and *Cascabela thevetia*

Asha Kumari[1] · Yash Vardhan Shukla[1] · Garima Chauhan[1] · Meghna Arya[1] · Monica Sharma[1]

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Abstract
Hydroxynitrile lyase enzymes are industrially important enzymes that are used to generate pure enantiomeric cyanohydrins in the fine chemical, pharmaceutical, and agrochemical industries. In nature, these enzymes are utilized by various organisms as a defence against herbivory or microbial attacks. Upon mechanical damage, cyanoglycosides or cyanolipids stored in different cell compartments come into contact with hydroxynitrile lyase and cause the release of a cyanide group, which further causes poisoning of the attacking agent. Due to their capacity to generate biologically active, stereoselective products, hydroxynitrile lyases (HNLs) enzymes are in high demand across various sectors. This study used the Feigl Anger test-based high-throughput technique and the HNL activity assay to detect cyanogenic plants containing novel HNLs. Sixty-five species of ornamental and weed plants were screened for cyanogenic activity and their potential to degrade racemic mandelonitrile. Out of all plants, ten exhibited racemic mandelonitrile degrading potential. Maximum hydroxynitrile lyase activity was observed in *Euphorbia mili* and *Cascabela thevetia,* further characterized by reaction conditions.

Keywords Hydroxynitrile lyase · Enantiomeric cyanohydrins · *Euphorbia mili* · *Cascabela thevetia*

Introduction

Hydroxynitrile lyases/ oxynitrilases (HNLs, EC 4.1.2.10, EC 4.1.2.46, EC 4.1.2.47, and EC 4.1.2.11) ubiquitously present enzymes and are actively involved in cyanogenesis in plant species that are cyanogenic and belong to Rosaceae, Gramineae, Linaceae, etc. Other than higher plants, this process is also present in bacteria, fungi, ferns, lichens, insects, and arthropods (Dadashipour and Asano 2011; Gleadow et al. 2000; Sharma et al. 2005), where these enzymes react with cyanoglycosides and release hydrocyanic acid (HCN) in injured tissue in response to pathogen attack or mechanical damage. Cyanide is released as a defence strategy against insects, fungal assaults, and herbivores (Nahrstedt 1985). Plants produce cyanogenic glycosides like amygdalin and prunasin as natural toxins of plants and store them in different tissues. On tissue disruption, cyanogenesis is started by β-glucosidase enzymes, which cleave cyanogenic glycosides to their corresponding cyanohydrin and carbohydrate, and later cyanohydrins are cleaved by HNL into the corresponding ketone/aldehyde and HCN (Dadashipour and Asano 2011). Besides cyanogenic plants, HNLs are also found in non-cyanogenic plants like *Arabidopsis thaliana* (Wöhler and Liebig 1837). Many superfamilies of HNLs have been identified so far, including the FAD-binding oxidoreductase, α/β hydrolase fold, dimeric α + β barrel, lipocalin-like folds, cupin, betv1-like folds, and Zn^{2+}-dependent alcohol dehydrogenase (Zheng et al. 2022). Except for the Zn2 + -dependent alcohol dehydrogenase superfamily, all superfamilies have at least one known HNL structural information.

Hydroxynitrile lyase is an important biocatalyst in chemical industries, where it is used in the synthesis of chiral cyanohydrins by exploiting reversible enzymatic reactions (Sharma et al. 2005). Cyanohydrins are biologically active precursor molecules that are utilized for the synthesis of compounds (α-hydroxy acids, β-amino alcohols, and α-hydroxyl ketones), and they find application in the fine chemicals, agrochemicals, and pharmaceutical industries

✉ Monica Sharma
dr.monikas@bbau.ac.in; monashimla@gmail.com

[1] Department of Biotechnology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India

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(Breuer et al. 2012; Sharma et al. 2005). The discovery and application of these enzymes serve as a great example of large-scale production, highlighting a few aspects of commercial biocatalysis. Due to the difficulty of producing enantiopure molecules efficiently, the chemical industries are either searching for novel enantioselective enzymes or designing enzymes to form enantioselective products (Song et al. 2023; Wiltschi et al. 2020). Although recombinant DNA technology has enabled the mass production of these enzymes for industrial applications, novel substrate specificity remains a challenge (Adrio et al. 2010). A solution to the problem is to detect various plant sources indicative of HNL activity; each of such plants may have a HNL with novel specificity for substrates, thereby partially tackling the second problem. Bioprospecting plants for new enantioselective enzymes are appealing because it can uncover new sources of HNLs with specific properties (Hernández et al. 2004; Asano et al. 2005). In this study, we have searched weeds (native plants) for HNLs because, in nature, weeds grow naturally and are not cultivated like food crops. They are plentiful due to higher seed setting, and they have also developed natural resilience against herbivory and insect attack through thousands of years of natural selection and adaptation (Clements et al. 2021). Weeds and a few ornamental plants produce thick waxy cuticle materials, toxic/repellent chemicals, cyanoglycosides, alkaloids, foul odour volatile chemicals, rancid compounds, etc., to reduce palatability (Charbonneau et al. 2018). We hypothesized that weeds and ornamental plants can be sources of novel HNLs. Therefore, we did bioprospecting of widely available plants to search for novel hydroxynitrile lyases. The plants possessing maximum HNL activities in *Euphorbia mili* (*E.* mili) and *Cascabela thevetia* (*C. thevetia*), were further characterized.

Material and methods

Sample collection

Sixty-five different weed plant and ornamental plant categories were selected as per their availability in the season, and abundance (Table 1). All samples were collected from the botanical gardens and campus of Babasaheb Bhimrao Ambedkar University (Lucknow) and adjoining areas in January. The samples were rinsed, and 50 mg of each was weighed and cut into small pieces. All chemicals used in the study were of analytical grade.

High throughput assay to detect cyanogenic activity

The Feigl-Anger test for cyanogenic activity was performed as a high-throughput assay (Takos et al., 2010; Feigl and Anger 1966). It works by oxidizing tetra base 4, 4-methylene bis (N, N-dimethylaniline) in the presence of HCN, which is formed as a byproduct of cyanogenesis. Following a single freeze–thaw cycle method of tissue disruption, the resulting product forms a distinct blue spot-on Feigl-Anger detection paper disc. Whatman No. 1 filter paper was cut into small discs to fit the microtiter plate wells. The detection paper disc was prepared by dipping Whatman No. 1 filter paper discs into a solution, which was prepared by combining two solutions having 75 mg of tetra base 4,4-methylene bis (N, N-dimethylaniline) and 75 mg of copper ethyl acetoacetate each in 7.5 ml of chloroform. The detection paper discs were soaked in the solution and dried. The dried paper discs were pale green and were kept at 4 °C in a dark, dry place until needed. The samples were cut into small pieces and placed on the microtiter plate; the Feigl-Anger detection paper discs were placed on the samples. The microtiter plate was covered with parafilm, and the lid was weighed down to prevent hydrogen cyanide diffusion from different wells. The sample tissues were permitted to thaw and disrupt at room temperature. The colour change was recorded every hour for up to 3 hours to detect the emission of any hydrogen cyanide. The negative control was distilled water, while the positive control was the apical bud of the rubber plant. A change in the detection disc's colour from pale green to blue signified a positive result. In contrast, no colour change indicated a negative result (Fig. 1). The images were recorded as soon as the colour change appeared, as the colour faded with time.

Crude enzyme preparation

With minor modifications, enzyme extract (crude) was produced according to Kassim et al. (2014) and Ueatrongchit et al. (2010). Apical leaves (1 g) were frozen with the help of liquid nitrogen and then crushed with a mortar and pestle to make a fine powder. It was then resuspended in 1 mL of 50 mM sodium citrate buffer (pH 5) and vortexed for 5–6 min. The subsequent slurry was centrifuged for 20 min at 10,000 g. The supernatant was collected as a crude enzyme extract. Using the Bradford method, protein concentration was determined by taking bovine serum albumin (BSA) as a reference (Bradford 1976).

HNL enzyme activity assay

The HNL activity was evaluated according to the method defined by Willeman et al. (2002) and Pratush et al. (2011). A reaction mixture of 5 mL with 0.1 M sodium citrate buffer (pH 5), 10 mM mandelonitrile and 100 µL of crude enzyme extract was used in the HNL activity assay. It was then incubated at 30 °C for 30 min; after that, it was quenched with 5 mL of trichloroacetic acid (TCA). The supernatant absorbance was measured at 280 nm after centrifugation (4000 × g). A unit of HNL activity is defined as the quantity

Table 1 List of plants screened for hydroxynitrilelyase activity using high-throughput Feigl-Anger test

Scientific Name	Type of Plant	Common name	Family	References
Abutilon indicum	Weed	Indian Abutilon	Malvaceae	Chanda et al. 2006
Aglaonema cochinchinense	Ornamental	Chinese evergreen	Araceae	Boyce et al. 2012
Allamanda blanchetii	Ornamental	golden trumpet	Apocynaceae	Gillman 2018
Allamanda cathartica	Ornamental	golden trumpet	Apocynaceae	Chiusoli and Boriani 1986
Alpinia zerumbet	Ornamental	Shell ginger	Zingiberaceae	Jackes et al. 2012
Alstonia scholaris	Ornamental	Blackboard tree	Apocynaceae	Patil 2019
Annona squamosa	Ornamental	Sugar-apples	Annonaceae	Dholvitayakhun et al. 2013
Begonia cucullata	Ornamental	Wax begonia	Begoniaceae	Pounders et al. 2015
Bougainvillea glabra	Ornamental	Bougainvillea	Nyctaginaceae	Cumo 2013
Bougainvillea spectabilis	Ornamental	Great Bougainvillea	Nyctaginaceae	Cumo 2013
Calendula arvensis	Ornamental	Field marigold, calendula	Asteraceae	Linnaeus 1863
Calendula officinalis	Ornamental	Pot marigold	Asteraceae	Lorraine 2012
Callistemon citrinus	Ornamental	Bottle brush	Myrtaceae	Gilman and Watson 1993
Calotropis gigantea	Weed	Madaar	Apocynaceae	Wang et al. 2009
Canna indica	Ornamental	Canna lily, Indian shot	Cannaceae	Henderson 2001
Cascabela thevetia	Ornamental	trumpet flower, lucky bean	Apocynaceae	Shannon et al. 1996
Cascabela thevetia (White)	Ornamental	trumpet flower, lucky bean	Apocynaceae	Shannon et al. 1996
Codiaeum variegatum	Ornamental	Gold dust croton	Euphorbiaceae	Huxley 1992
Codiaeum variegatum var. pictum	Ornamental	Eleanor Roosevelt croton	Euphorbiaceae	Huxley 1992
Coleus scutellarioides	Ornamental	Painted Nettle	Lamiaceae	Paton et al. 2019
Cordyline fruticosa	Ornamental	Broad leaf palm- lily	Asparagaceae	Hinkle 2007
Datura stramonium	Weed	Dhatura	Solanaceae	Henkel 1911
Dieffenbachia amoena	Ornamental	Dumb cane/Besarputih	Araceae	Croat 2004
Dieffenbachia seguine	Ornamental	Dieffenbachia/Dumb cane	Araceae	Croat 2004
Dracaena reflexa	Ornamental	Song of India	Asparagaceae	Gilmann 1999
Dracaena trifasciata	Ornamental	Snake plant	Asparagaceae	Lorraine 2012
Epipremnum pinnatum	Ornamental	Pothos, Money plant	Araceae	Govaerts 2015
Euphorbia cotinifolia	Ornamental	smoketree spurge	Euphorbiaceae	Nelson et al. 2007
Euphorbia milii	Ornamental	Crown of thorns	Euphorbiaceae	Huxley 1992
Euphorbia tithymaloides	Ornamental	Devil's backbone	Euphorbiaceae	Nellis 1997
Ficus benghalensis	Weed	Indian banyan	Moraceae	Bar-Ness 2010
Ficus elastica	Ornamental	Rubber plant	Moraceae	Wu et al. 2017
Ficus microcarpa	Ornamental	Chinese banyan, Malayan banyan	Moraceae	Shao and Zhao 2019
Grevillea robusta	Ornamental	Australian silky oak	Proteaceae	Menz et al. 2006
Hamelia patens	Ornamental	firebush	Rubiaceae	Lorea 2019
Helianthus annuus	Ornamental	Common sunflower	Asteraceace	Kartesz 2015
Hibiscus	Ornamental	China rose	Malvaceae	Lawton 2004
Jasminum sambac	Ornamantal	Mogra	Oleaceae	Marcel 1876
Jatropha curcas	Ornamental	Jatropha	Euphorbiaceae	Jules and Paull 2008
Justicia gendarussa	Weed	willow-leaved justicia	Acanthaceae	Agastian et al. 2006
Lantana camara	Ornamental	Putus	Verbenaceae	Day 2003
Lantana montevidensis	Ornamental shrub	Trailing Lantana	Verbenaceae	Sanders 2012
Lycium chinense	Weed	Lycium	Solanaceae	Potterat 2010
Murrayapaniculata	Weed	Orange Jasmine	Rutaceae	Mabberley 2022
Nephrolepsis cordifolia	Ornamental	fishbone fern	Nephrolepidaceae	Linnaeus 1836
*Nerium oleande*r	Weed	Oleander/Kaner	Apocynaceae	Lansdown 2013
Nyctanthes arbortristis	Ornamental	Night-flowering jasmine/Parijaat	Oleaceae	Saxena et al. 2002
Parthenium hysterophorus	Weed	Santa Maria/Gajarghass	Asteraceae	Oudhia et al. 1997
Philodendron hederaceum	Ornamental	Heartleaf philodendron	Araceae	Marderosian et al. 1976
Plumeria obtusa	Ornamental	Graveyard Flower/Gulachin	Apocynaceae	Bihani et al. 2021

Table 1 (continued)

Scientific Name	Type of Plant	Common name	Family	References
Ricinus communis	Ornamental	Castor	Euphorbiaceae	Tan et al. 2009
Sambucus canadensis	Weed	Common elderberry	Adoxaceae	Oke 1969
Sanchezia speciosa	Ornamental	Zebra Plant	Acanthaceae	Leonard and Smith 1964
Saraca indica	Ornamental	Ashok tree	Fabaceae	Zuijderhoudt 1968
Senna occidentalis	Weed	Antbush	Fabaceae	Vashishtha et al. 2007
Solanum americanum	Weed	Common nightshade	Solanaceae	Turner and Aderka 2009
Sonchus asper	Weed	Rough milk thistle	Asteraceae	Humbert 1923
Sonchus oleraceus	Weed	Prickly Sowthistle	Asteraceae	Nyerges 2017
Spathiphyllum cochlearispathum	Ornamental	Peace lily	Araceae	Gustav and Engler 1879
Syngonium podophyllum	Ornamental	Arrowhead plant	Araceae	Lorraine 2012
Tabernaemontana divaricata	Ornamental	Pinwheel flower	Apocynaceae	Lakhey and Pathak 2020
Tecoma stans	Ornamental	Yellow bells	Bignoniaceae	Anand, and Basavaraju 2021
Thuja occidentalis	Ornamental	White cedar/Morpankh (Hindi)	Cupressaceae	Johnston 1990
Tradescantia fluminensis	Weed	Small-leaf spiderwort	Commelinaceae	Kelly and Skipworth 1984
Tradescantia spathacea	Ornamental	Oyster plant	Commelinaceae	Hunt 1994

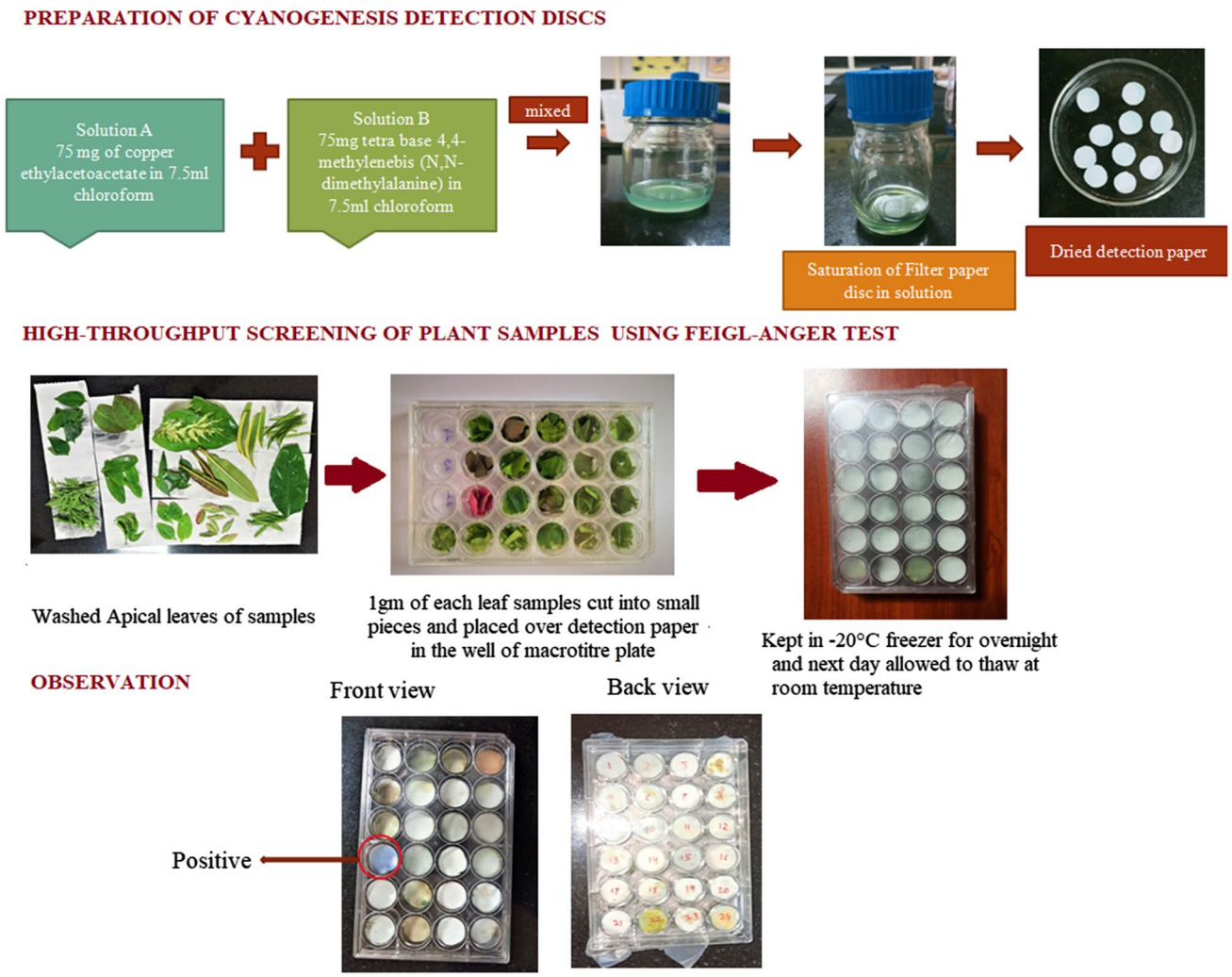


Fig. 1 The Feigl-Anger test is a novel rapid method for high-throughput screening of samples based on the preparation of cyanide detection discs

of enzymes that catalyse the release of micromoles of benzaldehyde produced per minute per mg of protein under the assay conditions.

Qualitative assay for HNL activity using HPLC

HPLC was performed to detect the formation of benzaldehyde using the UFLC C-18 column and Shimadzu UFLC system (DGU-20A5R Degassing unit; LC-20AD prominence liquid chromatography; SIL-20AC HT prominence auto sampler; CTO-10AS VP column oven; RID-20A refractive index detector; SPD-20A UV/VIS detector; CBM-20A communications bus module). The sample was analysed using (65% v/v) acetonitrile in water as the mobile phase at a flow rate of 1.0 ml/min at ambient temperature. The absorbance was recorded at 210 nm and 280 nm to detect the formation of benzaldehyde.

Reaction conditions optimisation for HNL activity assay:

Reactions were carried out using various buffer systems (sodium citrate buffer, potassium phosphate buffer, and glycine buffer of 0.1 M strength), a buffer with pH (3–10), temperature (20 °C–50 °C), incubation time (10 min–60 min), and substrate concentration (10 mM–100 mM) for the assay of hydroxynitrile lyase activity of *E. milli* and *C. thevetia* (White).

Statistical analysis

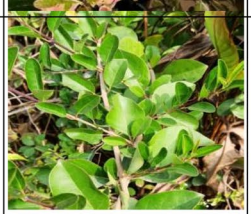
All the data was processed using Excel for data analysis and graphical illustrations. All reactions were conducted in a set of triplicates, and the values were reported as mean ± S.D. To compare variance and statistical analysis between *E. mili* and *C. thevetia* HNL activity due to the impact of reaction conditions, a two-way analysis of variance (ANOVA) with replication was used. Differences in the means of the characteristics under consideration were considered significant when $p < 0.05$.

Result and discussion

Based on the Feigl Anger test, ten plants were identified to possess the cyanogenic activity of sixty-five plants, and the HNLs assay confirmed the presence of hydroxynitrile lyase activity (Table 2). The Feigl-Anger high throughput screening approach is the simplest way to detect plants for cyanogenic activity, which may be confirmed with enzyme assay (Tomescu et al. 2020). The use of the fresh material available in the field eliminates substantial enzyme degradation caused by time, shipping constraints, and improper storage. It also reduces the requirement for large volumes of plant tissue samples, allowing for a more focused collection of possible HNL candidates capable of catalyzing the breakdown of cyanohydrin substrates into HCN and aldehyde or ketone (Kassim et al. 2014). The highest HNL activity was found in the leaves of *E. mili* (1.133 U) (Huxley 1992), followed by *C. officinalis* (0.256 U) (Lorraine 2012) and *C. thevetia* (White) (0.200 U) (Shannon et al. 1996). Because the activity of hydroxynitrile lyase is frequently low in crude leaf homogenates, the specific activities evaluated in this investigation were not surprising (Kassim et al. 2014). Since *C. officinalis* is only available in winter, its HNL activity was not characterised and discussed in this paper. *E. mili* exhibited maximum activity in potassium phosphate buffer. The buffer potassium phosphate was demonstrated to be appropriate for *C. thevetia*. The activity of HNL from *E. mili* was highest (7.83 U) at pH 7 of potassium phosphate buffer. HNL of *C. thevetia* also exhibited maximum activity (1.25 U) at pH 7 of potassium phosphate buffer (Fig. 2). The pH of the plant macerate also has a significant impact on HNL activity (Dadashipour and Asano 2011). From the seeds of *Eriobotrya japonica*, Ueatrongchit et al. (2008) discovered and homogeneously purified HNL. The specific activity of the crude extracts was 0.8 U/mg, but following purification using the Concanavalin A Sepharose 4B affinity column, it increased to 40.9 U/mg. Regarding incubation duration, the greatest HNL activity of *E. mili* was observed at 20 min (7.835 U), while *C. thevetia* was observed at 30 min (3.492 U) with varied time intervals of 10 min up to 60 min (Fig. 3). HNL activity was greatest in *E. mili* at 25 °C (22.47 U) and in *C. thevetia* at 20 °C (2.251 U), with activity decreasing as temperature increased (Fig. 4). Maximum HNL enzyme activity of *E. mili* is at 30 mM (25.20 U), while *C. thevetia* is also at 30 mM (3.181 U) (Fig. 5). HPLC was performed as a qualitative assay for detecting the HNL-mediated formation of benzaldehyde. Both *E. mili* and *C. thevetia* samples showed the formation of the benzaldehyde, and a major peak was observed at RT ~ 2.96 min at 210 nm and RT ~ 4.67 min at 280 nm (for *E. mili*) and RT ~ 2.855 min at 210 nm and at RT ~ 4.675 min at 280 nm (for *C. thevetia*) (Supplementary data).

No properties of cyanogenesis or HNL activity have been mentioned for any of the chosen plants in the literature, and this is the first time we are reporting these new sources of HNLs. Statistical analysis using two-way ANOVA with replication showed that the F value (Fisher statistics value) were much higher than the F-crit values for all the variables for hydroxynitrile lyase of *E. mili* and *C. thevetia,* meaning that all null hypothesis are rejected (H1: means of observation grouped by one factor are the same; H2: means of observation grouped by the other factor are the same; H3: there is no interaction between the two factors), and both HNLs follow the alternate

Table 2 List of plants exhibiting hydroxynitrile lyase activity in the crude enzyme extracts prepared from leaves of the plants

Leaf sample	CommonName; Family	Image	Protein concentration mg/ml	Specific activity mM/mg/min
Calendula officinalis (Lorraine 2012)	Potmarigold; Asteraceae		0.26	**0.256**
Cascabela thevetia (White) (Shannon et al. 1996)	Trumpetflower/ lucky bean; Apocynaceae		2.26	**0.200**
Cordyline fructicosa (Hinkle 2007)	Broadleafpalm lily; Asparagaceae		0.94	0.127
Dieffenbachia seguine (Croat 2004)	Dieffenbachia/ dumb cane; Araceae		2.55	0.016
Dracaena trifasciata (Lorraine 2012)	Snake plant; Asparagaceae		1.20	0.057
Euphorbia milli (Huxley 1992)	Crown of thorns;Euphorbiaceae		0.67	**1.133**
Euphorbia tithymaloides (green) (Nellis 1997)	Devil'sbackbone; Euphorbiaceae		1.84	0.038
Euphorbia tithymaloides (redwhite) (Nellis 1997)	Devil'sbackbone; Euphorbiaceae		0.83	0.110
Lycium chinense (Potterat 2010)	Lycium; Solanaceae		2.12	0.042
Senna occidentalis	Antbush; Fabaceae		5.62	0.066

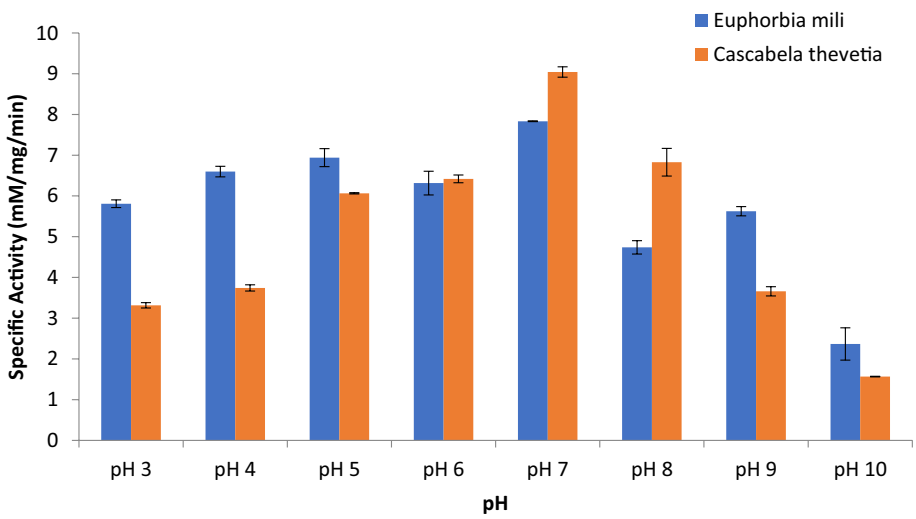


Fig. 2 Effect of pH on the activity of the HNL enzyme extracted from *E. mili* and *C. thevetia.* (All reactions were run in triplicates and represented at mean ± Standard deviation)

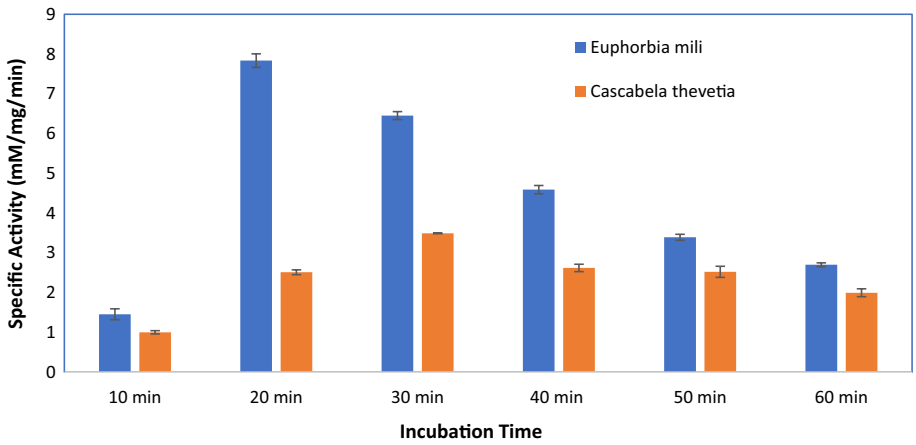


Fig. 3 Effect of incubation time on the activity of hydroxynitrile lyase (HNL) enzymes extracted from *E. mili* and *C. thevetia* for the degradation of mandelonitrile (All reactions were run in triplicates and represented at mean ± Standard deviation)

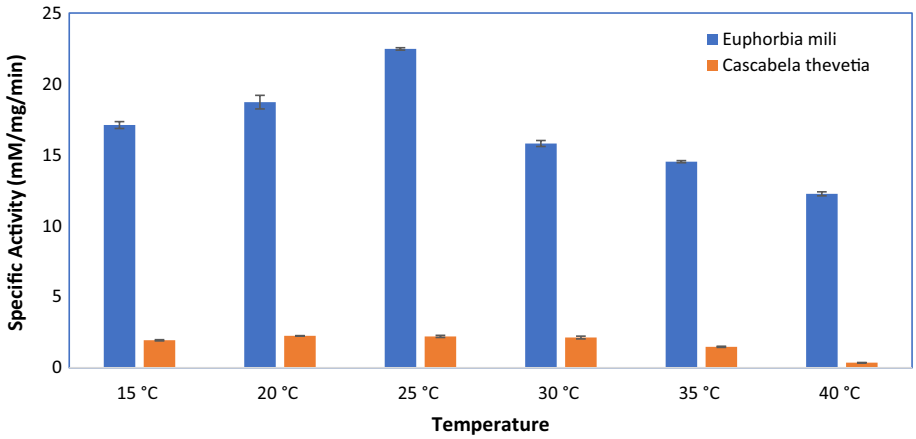


Fig. 4 Effect of temperature (from 15 °C–45 °C) on the activity of HNLs of *E. mili* and *C. thevetia* for the degradation of mandelonitrile (All reactions were run in triplicates and represented at mean ± Standard deviation)

hypothesis and are not related to each other and different in nature (Supplementary data). While the p-values for the specific activities of *E. mili* and *C. thevetia* for all variables were less than 0.05, showing that the particular activity of the hydroxynitrile lyase of *E. mili* and *C. thevetia* is statistically significant (Supplementary data).

Even though the families evaluated in this study comprise cyanogenic species, some of the chosen plants did not display cyanogenic activity. It is imperative to remember that some plants contain cyanogenic polymorphism, which means they have quantitative variation in the concentration of endogenous cyanogenic chemicals and glucosidase

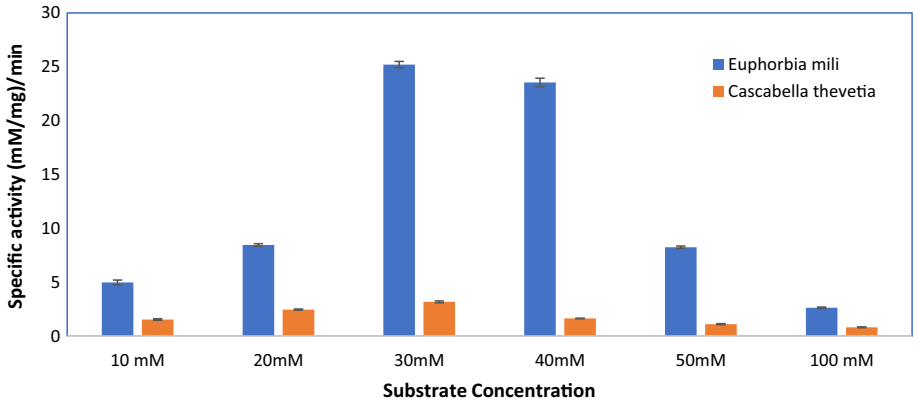


Fig. 5 Optimization of substrate concentration (10–100 mM) for the HNL enzyme activity extracted from *E. mili* and *C. thevetia* for the degradation of mandelonitrile. (All reactions were run in triplicates and represented at mean ± Standard deviation)

(Goodger and Woodrow 2002). The absence of cyanogenic activity in plants does not always mean they are not cyanogenic. The age/stage of plant growth and development, the section or part that was evaluated, seasonal change, environmental circumstances, and climate may have influenced the study results. The study was based on the apical leaves of the selected plant because they are known to have the highest concentration of cyanogenic glycosides (Gleadow and Woodrow 2000).

Seasonal variation in hydroxynitrile lyase activity was also observed in our study (data not included). Gleadow and Woodrow (2000) reported that with maturation, the cyanogenic glycoside content reduces. Hernandez et al. (2004) found that the same plant's various sections (leaves and seeds) produced varied results. They discovered that while the leaves of some plants were not cyanogenic, their seeds were, and vice versa. They also found that the amount of cyanogenic glycoside in immature leaves varies depending on the season. They hypothesised that as the season changes, the concentration of cyanogenic glycoside changes, which is also caused by the amount of soil nitrogen, temperature, climate, and other factors. Gebrehiwot and Beuselinck (2001) used field and greenhouse studies to validate seasonal variations in HCN concentrations. Plants in the summer and spring had 50% higher HCN concentrations than those in the autumn or winter, with the winter having the lowest levels. It is well known that the quantity and concentration of certain compounds in plants vary depending on the season and age (Pichersky and Lewinsohn 2011).

Conclusion

This study revealed two novel cyanogenic plants with hydroxynitrile lyase activity. The number of plants known to contain hydroxynitrile lyase activity is increased by these findings, and these plant enzymes may also have novel characteristics like substrate selectivity and enantioselectivity. Due to seasonal variance, the plants that remained and tested negative may not be guaranteed as non-cyanogenic. In future work, the HNLs from these two plants will be heterologously expressed in a microbial host.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42535-024-00949-6.

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Author contribution AK: Writing-original draft, Investigation, Methodology. YVS-Investigation assistance and sample collection, GC and MA: Writing review and editing. MS**:** Conceptualization, Supervision, Editing, Validation, Writing- Review & Editing.

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Data availability All data supporting this research will be made available on request.

Declarations

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

Ethical approval Not applicable.

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