



Turning Waste into Beneficial Resource: Implication of *Ageratum conyzoides* L. in Sustainable Agriculture, Environment and Biopharma Sectors

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Abstract

The annual herb, *Ageratum conyzoides* L. (Asteraceae), is distributed throughout the world. Although invasive, it can be very useful as a source of essential oils, pharmaceuticals, biopesticides, and bioenergy. However, very limited information exists on the molecular basis of its different utility as previous investigations were mainly focused on phytochemical/biological activity profiling. Here we have explored various properties of *A. conyzoides* that may offer environmental, ecological, agricultural, and health benefits. As this aromatic plant harbors many important secondary metabolites that may have various implications, biotechnological interventions such as genomics, metabolomics and tissue-culture can be indispensable tools for their mass-production. Further, *A. conyzoides* acts as a natural reservoir of begomoviruses affecting a wide range of plant species. As the mechanisms of disease spreading and crop infection are not fully clear, whole-genome sequencing and various advanced molecular technologies including RNAi, CRISPER/Cas9, multi-omics approaches, etc., may aid to decipher the molecular mechanism of such disease development and thus, can be useful in crop protection. Overall, improved knowledge of *A. conyzoides* is not only essential for developing sustainable weed control strategy but can also offer potential ways for biomedical, environment, safe and clean agriculture applications.

Keywords *Ageratum conyzoides* · Agri-biotechnology · Biopharma · Crop protection · Clean environment · Genome editing · Multi-omics · NGS · RNAi · Secondary metabolites

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid	GC-FID	Gas chromatography equipped with flame ionization detector
AEV	<i>Ageratum enation virus</i>	GC-MS	Gas chromatography-mass spectrometry
AYLCB	<i>Ageratum yellow leaf curl betasatellite</i>	GTI	Growth tolerance index
AYVV	<i>Ageratum yellow vein virus</i>	HPLC-DAD	High-performance liquid chromatography with diode array detector
BAP	6-Benzyl aminopurine	HPLC-HRMS	High-performance liquid chromatography-high-resolution mass spectrometry
CRD	Completely randomized design	IAA	Indole-3-acetic acid
		IBA	Indole-3-butyric acid
		ISSR	Inter simple sequence repeats
		LC-MS	Liquid chromatography-mass spectrometry
		MS	Murashige and Skoog
		MYMIV	Mungbean yellow mosaic India virus
		NAA	α -Naphthalene acetic acid

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NGS	Next-generation sequencing
NTSR	Non-targeted-site resistance
PM	Point mutation
qRT-PCR	Qualitative real-time polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphisms
SGS	Single-gene sequencing
SSR	Simple sequence repeats
TLC	Thin-layer chromatography
TSR	Target-site resistance
TYLCTZV	Tomato yellow leaf curl Tanzania virus
UPLC-PDA-ESI-QToF-MS	Ultra performance liquid chromatography, coupled to photodiode-array and electrospray ionization/quadrupole-time-of-flight mass spectrometry

Introduction

Ageratum conyzoides L., an important medicinal herb from the *Asteraceae* family, is native to Central America but has been found globally including Africa, Asia, and South Pacific Islands [1]. The plant has different names as per the language such as Goat weed (English), Visadodi (Hindi), Visamustih (Sanskrit), Mejorana (Spanish), Mentrasto (Portuguese), Bhedaa Jhaar (Nepali), and Uralgidda (Kannada). The genus name *Ageratum* is based on the Greek word ‘*a geras*’ (means non-aging), while the species name *Conyzoides* is through ‘*konyz*’ (as it looks similar to the *Inula helenium* L.) [2]. The plant usually grows close to habitation and prospers best in soils with high nutrients, minerals, and moisture content [3]. It is very frequently found in and around waste places, gardens, grasslands, disturbed habitats, forest edges, watercourses, ruined sites, etc., covering from sea level to mountain. With a height of 1–2 m, this aromatic herb shows annual, erect, branched, slender, and hairy features. White hairs are found on the leaves and stems. Leaves are simple with petiolate, ovate, or rhomboid-ovate shapes with a size range of 1–10 cm × 0.5–7.0 cm, apex acute, and length up to 7.5 cm [4, 5]. Generally, the flower color is white to purple with an inflorescence arrangement of a close terminal type. The achene-type fruits are effortlessly dispersed. The seeds having photoblastic nature may often lose within a year [6]. Although about 30 species have been reported from the *Ageratum* genus, the number of phytochemically characterized species is low. The essential oils obtained from *A. conyzoides* contain hydrogen cyanide and

coumarin that may contribute to a powerful nauseating odor [7]. The plant has been mainly studied for its biological activities along with potential medicinal applications and earlier reviews have focused largely on pharmacological activities from *A. conyzoides* [4, 8–10]. However, the current study explores various ways of modern biotechnological interventions by which it can offer solutions for a better environment, ecology, health, and/ or agriculture. The demand is growing on crop breeding for improved resilience against abiotic and biotic stress with changing climatic conditions globally. Based on various biological and chemical properties, this weed plant might be potentially turned into a beneficial commodity while addressing important questions such as (1) how can we utilize it for the sustainable environment, agriculture, and also the industrial sector? (2) How it can be used in agricultural waste management? (3) How modern molecular and tissue-culture techniques can be applied for its mass multiplication and production of important secondary metabolites with potential utility in safeguarding agriculture, health, or the environment? (4) How can we exploit modern biotechnological tools to prevent the spread of disease from *A. conyzoides* and thus, can utilize this knowledge to generate resistant crops?

The Usefulness of *A. conyzoides* in Agriculture, Environment, and Industrial Utilities

Ageratum conyzoides successfully invades native habitats due to its fast growth rates, short life cycles, drought tolerance, allelopathy, greater competitive abilities, and higher reproductive potential [11]. Moreover, seed germination is the most important stage in the plant life cycle that contributes to its distribution. As per the surrounding environment, *A. conyzoides* has a wide range of temperature and light suitability to allow seed germination, which helps them to adapt to different local micro-habitats [12]. On the other side, the germination and growth rates of *A. conyzoides* were severely reduced in an experimental study by the application of different concentrations (50, 100, and 200 M) of parthenin (a sesquiterpene lactone from *Parthenium hysterophorus* L.), while it was completely inhibited at 400 M level [13]. The parthenin exhibited germination and growth reduction effects by altering the contents of various macromolecules (proteins, carbohydrates, chlorophyll, etc.) and specific activities of important enzymes such as protease, α -amylase, and β -amylase.

Further, novel genetic resources with higher yield and capacity to withstand the invasive species during climate change scenarios can be developed based on the advanced knowledge from the genetic exploration on the causal physiological processes, which offer competitive advantages to

weed plants [14]. For instance, a study revealed that the average number of plant species and diversity was reduced by 32.10% and 41.21%, respectively, due to the weed invasion at Shivalik hills of Hamirpur district in Himachal Pradesh, India. *A. conyzoides* invasion significantly reduced the productivity and diversity of native species [5]. Moreover with a nutrient gradient, phenotypic plasticity, growth, and functional traits of *A. conyzoides* and *Eupatorium catarium* Veldkamp were analyzed under two contrasting conditions. The study revealed similar biomass of these two species under low-nutrient treatment with non-competitive condition. However, the addition of nutrients led to increased biomass in them. Consequently, under high-nutrient treatment, *E. catarium* and *A. conyzoides* had better growth over *Vernonia cinerea* (L.) Less. (a native species) in both competitive and non-competitive conditions [15]. On the other side, these two invasive species and the native plant exhibited similar growth during comparatively low nutrients in soil despite having sufficient water and light availability [16]. Thus, based on the surrounding environmental conditions *A. conyzoides* can potentially affect growth as well as plant diversity. Consequently, superior knowledge of various attributes of *A. conyzoides* is vital to develop better ways of weed control. It can potentially be useful as a source of essential oils, pharmaceuticals, biopesticides, and bioenergy with different applications (Fig. 1) [17]. Its allelochemicals are potential natural pesticides, which can offer a better solution for weed and pest management [16]. This plant otherwise can also be used as organic material to improve soil nutrient levels. The *A. conyzoides* was applied as Bokashi (100, 120, 140, 160 g/ polybag in a test and control of 0.6 g NPK/ polybag) to determine their nutrient and growth effects on tomato. The Bokashi of Bandotan (*A. conyzoides*) 120 g/ polybag exhibited the greatest effect on the tomato weights.

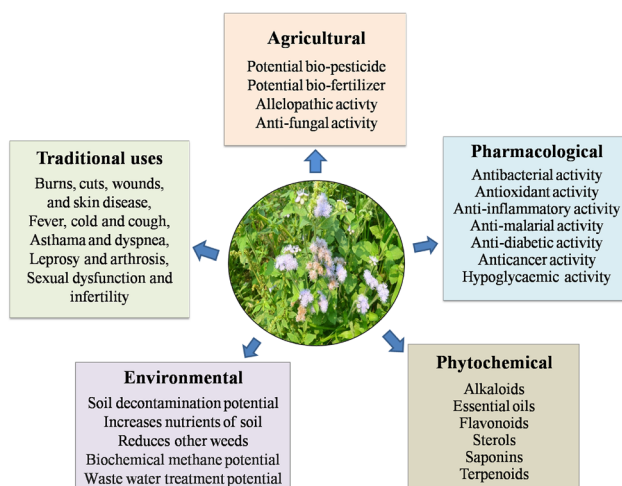


Fig. 1 Various important activities / applications of *A. conyzoides*

However, there were no effects on the biomass, height, wet weight, as well as nutrient contents (vitamin A and C) of tomato [19]. Extrinsic environmental variables (total soil nitrogen, and organic matter) and the evolutionary structure of the resident community significantly affected the diversity of *A. conyzoides* [20].

Phytopathogens (e.g., fungi, bacteria, viruses) cause various diseases to crops and lead to a decline in one-third of global agriculture production [21]. To mitigate this loss, different chemicals/ fertilizers (viz. fungicides, pesticides, etc.) are used which could have severe toxic effects on human beings as well as on surrounding environment [22]. Subsequently, synthetic and chemical fertilizers take a longer time to be degraded in soil compared to biofertilizers. Extracts of many plants including invasive species are known to exhibit allelopathic properties and can be utilized in agriculture as biofertilizers. The active ingredients found in these plants can be used in the form of extracts or can also be synthesized. Plant extracts can have a low environmental impact due to their fast degradation in soil [23]. For example, the suppressive effects of *A. conyzoides* were evaluated on the growth and germination response from radish (*Raphanus sativus* L.) and paddy weeds [*Echinochloa crus-galli* (L.) P. Beauv., *Monochoria vaginalis* (Burm.f.) C. Presl and *Aeschynomene indica* L.]. In comparison to *A. conyzoides* root and stem tissue, leaves (2 t/ha input) showed significant suppression with ~70% growth reduction of *E. crus-galli* while completely prevented the germination of *M. vaginalis* and *A. indica* in calcareous soil conditions [24]. Similar treatment (2 t/ha) with leaves from *A. conyzoides* on a rice (*Oryza sativa* L. var. *indica*) field led to an 86% decrease in paddy weeds. This not only significantly lowered the fresh and dry weights (~75% inhibition) of these weeds but also contributed to a 14% higher rice yield in comparison to the butachlor application [24]. On the other side, *A. conyzoides* extract (ACE) was found to reduce the growth (e.g., plant height, number of branches and leaves) and development of redroot amaranth (*Amaranthus retroflexus* L.), peanut (*Arachis hypogaea* L.), cucumber (*Cucumis sativus* L.), ryegrass (*Lolium multiflorum* Lam.), and rice (*Oryza sativa* L.) by releasing water-soluble phytochemicals (e.g., precocenes, 2H-benzopyran, monoterpenes, and sesquiterpenes) [25, 26]. Apart from this, *A. conyzoides* leaf biomass was found to increase the microbial enzymatic activities, waste mineralization and microbial population build-up, earthworm growth, and fecundity when applied to a vermicomposting bed at 50–75% proportion along with cow dung. Compost extracts enhanced the soil respiration rate and the germination index of mustard (*Brassica campestris* L.), indicating the suitability of *A. conyzoides* as a potting media for vermicomposting [27]. Thus, *A. conyzoides* might be considered as a promising natural growth promoter, herbicide

and can offer valuable solutions for sustainable and safe agriculture.

For a Clean Environment

Increasing population density and climate change along with inefficient management of water resources have led to water scarcity all over the globe, and the need of the hour is to develop an efficient water harvesting system utilizing solar energy and re-using wastewater [28]. The wastewater is widely used in agricultural fields in peri-urban areas of developing countries [29]. However, the wastewater used in agriculture for irrigating crops contains harmful pathogens, bacteria, viruses, excessive nitrogen, phosphorus, and heavy metals, which are also deleterious to humans and animals [30]. Wastewater treatment plants used in urban areas are very costly and often yield partially treated wastewater that may have still some contaminants [31]. To overcome this, constructed wetlands that mimic the functions of natural wetlands can be used to treat wastewater naturally involving wetland vegetation and associated microbial populations that can uptake excessive nitrogen, phosphorus, and heavy metals [32]. Several wetland plants such as *Typha latifolia* L., *T. angustifolia* L., *Schoenoplectus validus* (Vahl) Á. Löve & D. Löve, *Phragmites australis* (Cav.) Trin. ex Steud., *Juncus effusus* L., *Canna indica* L., *Eichhornia crassipes* (Mart.) Solms, and *Lemna minor* L. have the potential to remove contamination of nitrogen and phosphorus from wastewater [33, 34]. More recently, *A. conyzoides* has indicated similar efficacy in combination with wetland plants. The study has demonstrated effective removal of excessive phosphorus, nitrogen, and fecal coliforms from domestic wastewater using *A. conyzoides* along with *Pistia stratiotes* L., *T. latifolia*, and *C. indica* [35]. Additionally, municipal and electronic wastes can lead to more soil pollution, which is a grave universal issue for waste sites and related environments [36]. The phytoremediation approach can offer a potential strategy to protect the soil environment from such contamination [37, 38]. As *A. conyzoides* can grow easily on contaminated soil, it can take up heavy metals from any waste site [36]. Soil decontamination potentiality of *A. conyzoides* was evaluated in pot experiments using ethylenediaminetetraacetic acid (EDTA at 0.1 g/kg) in combination with kinetin (100 µM). Leaves exhibited the highest accumulation of Fe (6.51–38.58%), Mn (0.14–73.12%), Zn (5.24–269.07%), and Cu (9.38–116.59%), whereas accumulation of Pb (22.83–113.41%) and Cr (21.05–500%) was highest in the stem, as compared with controls. Plants exhibited overall improved growth with the planned kinetin-EDTA combination [36]. Similarly, another weed

Santa-Maria (*P. hysterophorus* L.) can also reduce heavy metals from contaminated soil [39].

For Industrial Purpose

The *A. conyzoides* extract (ACE) can be used to manufacture better sodium alginate (SA) films having improved physical, mechanical, and thermal assets [40]. With ACE, the tensile strength was superior and the water vapor transmission rate was significantly reduced in SA film. Consequently, the thermal stability and swelling rate of the SA film were also better. Thus, ACE-SA film can potentially serve as an effective wound-dressing material [40]. Additionally, the biochemical methane potential (BMP) of *A. conyzoides* has been studied to find the ideal food to microorganism (F/M) ratio and as an alternative energy source [41]. The assay revealed that out of different ratios examined (1.0, 1.5, 2.0, and 2.5), the F/M ratio of 2 showed maximum methane (CH₄) and volatile solid (VS) production from the anaerobic digestion. Further, the highest biogas production was achieved with 205 ± 10 mL CH₄/g VS and cumulative methane production reached up to 4994 ± 25 mL on the 25th day. Within 30 days of incubation, 80% of biogas production was achieved and kinetic study also confirmed the efficiency of biogas production [41]. Thus, the biomass from this terrestrial invasive plant can be converted as a very effective resource and used in an eco-friendly manner with the generation of viable clean energy at a minimum cost.

Effectiveness of Secondary Metabolites from *A. conyzoides*

The chemical composition of *A. conyzoides* has been analyzed by various qualitative and quantitative methods such as gas-chromatography-mass spectrometry (GC–MS), GC-equipped with flame ionization detector (GC–FID), liquid-chromatography-mass spectrometry (LC–MS), thin-layer chromatography (TLC), high-performance liquid chromatography-high-resolution mass spectrometry (HPLC–HRMS), HPLC with diode array detector (HPLC–DAD), and ultra-performance liquid chromatography, coupled to photodiode-array and electrospray ionization/quadrupole-time-of-flight mass spectrometry (UPLC–PDA–ESI–QToF–MS). These have allowed the identification of various phytochemicals, such as the pyrrolizidine alkaloids, saponins, coumarin, pyrrolone, phenolic acids, polymethoxyflavones, and terpenoids [8, 42–44]. *A. conyzoides* contains many different types of sterols like brassicasterol, β-sitosterol, cholesterol, stigmasterol, spinasterol, etc. [45, 46]. Similarly, it is also very rich in flavonoids such as scutellarein-5,6,7,1-tetrahydroxyflavone, polymethoxy flavones, eupalestin, quercetin, kaempferol, kaempferol 3,7-diglucopyranoside,

(2S)-7,3',4'-trimethoxyflavone, (2S)-7-methoxy-3',4'-methylenedioxyflavan, 5,6,7,3',4',5'-hexamethoxyflavone, nobiletin, and 5'-methoxynobiletin [43, 47, 48]. Earlier from the stems, a new isoflavone glycoside, [5,7,2',4'-tetrahydroxy-6,3'-di-(3,3-dimethylallyl)-isoflavone-5-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranoside] was detected [49]. Glycosidal flavonoids isolated from ethyl extracts are *p*-hydroxybenzoic acid, quercetin-3-O-rhamnopyranoside, and quercetin-3,7-diglucopyranoside [24, 50, 51]. Additionally, GC-MS analysis of *A. conyzoides* essential oils are reported to contain 7-methoxy-2,2-dimethylchromene (precocene I), β -copaene, hexanal, trans-cadina-1(6), ageratochromene (precocene II), α -calacorene, caryophylla-4(12), germacrene-D, trans-cadina-1(6),4-diene, 8(13)-diene-5- β -ol, β -caryophyllene, α -caryophyllene, trans- β -farnesene, β -cubebene, coumarin, phytol (a diterpene alcohol) and 1,10-di-epi-cubenol [52–54]. Additionally, a total of 51 constituents including chromenes (85.2%), chromans (0.9%), oxygenated monoterpenoids (1.4%), phenylpropanoids and benzenoids (2.33%), oxygenated sesquiterpenoids (0.8%), monoterpenoid hydrocarbons (5.0%), and sesquiterpenoid hydrocarbons (4.3%) have been reported from the leaves of *A. conyzoides* [55]. Similarly, A new chromene, [2,2-dimethylchromene-7-methoxy-6-O- β -D-glucopyranoside], has also been identified from the total plant ethanol extract [56].

Many of these compounds can be useful in different ways (Fig. 2) such as a substitute for synthetic and chemical fungicides [57, 58]. Soil pathogenic fungi and weed invasion are major threats to citrus plants as they significantly reduce

the yield. The invasion of other weeds and pastes can be controlled by incorporating *A. conyzoides* plants in the citrus orchards [59]. Three flavones and ageratochromene allelochemicals (levels of 11 to 93 μ g/ g of soil) were released from *A. conyzoides* on citrus orchards that significantly inhibited (47.3% to 71.2%) the development of three weeds (*Cyperus difformis* L., *Bidens pilosa* L., and *Digitaria sanguinalis* (L.) Scop.). This also prevented the growth of disease-causing soil fungi, namely *Fusarium solani* Mart. (Sacc.), *Pythium aphanidermatum* (Edson) Fitzpatrick and *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.). This finding has suggested that the incorporation of *A. conyzoides* and isolated allelochemicals (flavones and ageratochromene) in soil may be useful in controlling other invasive weeds and phytopathogens [59]. Similarly, the aqueous, *n*-hexane and methanol extracts from various tissues of *A. conyzoides* at different concentrations (2, 4, and 6 w/v) were used to assess the antifungal potential against pathogenic fungi *Fusarium solani*. All the extracts exerted antifungal activity and significantly repressed the growth of the fungi [60]. Another such study has been recently performed on *Puccinia arachidis* Speg., which is the causal organism of rust disease in peanuts. Crude ACE was very effective against this pathogen when used at 2.5% to 5.0% concentrations. Additionally, the aqueous ACE showed maximum antifungal activity against *Aspergillus niger* Tiegh. and *A. ustus* (Bainier) Thom & Church with an average inhibition zone of 20 mm, while the minimum inhibition was recorded against *A. fumigatus* Fresen. with 7 mm at 800 mg/mL concentration [57]. This indicates that it can also be an effective biofungicide and thus, helps to reduce the use of synthetic fungicides [58]. Taken together, *A. conyzoides* is very rich in different compounds such as sterols, flavonoids, saponins, chromenes, pyrrolizidine, alkaloids, coumarin, pyrrolone, terpenoids, and lignin, and thus, can have potential implications in different sectors for a safe environment, agriculture, and biomedicine (Fig. 2).

Essential oils from *A. conyzoides* can also be a good botanical insecticide and may be useful against insects for integrated pest management practices. These secondary metabolites such as alkaloids, flavonoids, phenols, and tannins possibly can be exploited for the development of natural pesticides and controlling pests for sustainable crop production [18]. For instance, using these oils, in vitro and in vivo fumigant tests resulted in 100% mortality of *Tribolium castaneum* (Herbst), the storage grain insect. *A. conyzoides* essential oils totally destroyed the insects at 1000 ppm. On the other side, these oils were non-phytotoxic; and did not affect the seed germination and growth of the seedlings [61]. Similarly, different concentrations (0.1, 0.2, 0.5, and 1.0%) of petroleum ether leaf extract were used to evaluate insecticidal activity against the *Epilachna 28 punctata* larvae. The 1.0% and 0.5% of leaf ACE exhibited 100% and 66.67%

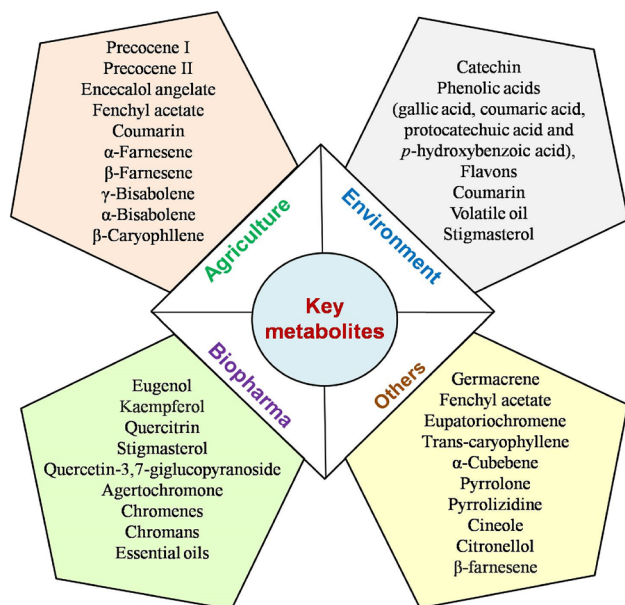


Fig. 2 Potential metabolites from *A. conyzoides* having implication in different areas

mortality of the larvae, respectively. Both 0.1% and 0.2% concentration indicated 33.33% mortality [62]. Additionally, the whole plant ACE when used in different doses (2, 4, 6, 8, and 10% conc.) to assess the potential as a biopesticide against pest of Caisim (*Brassica juncea* L. Czern.), 4% level was the most effective botanical pesticide to control an amount of pest in Caisim compared to the treatment without pesticides [63]. In another recent study, methanolic leaf ACE (0, 6, 8, 10, and 12% conc.) was used as a biopesticide against caterpillar larvae (*Spodoptera litura* Fabricius). Larvae mortality rate was significantly improved with the increased ACE concentration with the highest mortality at the 12% level [64]. Antifungal bioassays using various fractions of stem ACE such as *n*-hexane, chloroform, *n*-butanol, and ethyl acetate were used against disease-causing pathogen *Macrophomina phaseolina* (Tassi) Goid. All fractions exhibited a reduction in pathogen biomass over the control. Several compounds from ACE [such as 2H-1-benzopyran, 7-dimethoxy-2,2-dimethyl, hexadecanoic acid, 11-octadecanoic acid, methyl ester, 1,2-benzenedicarboxylic acid, and mono(2-ethylhexyl) ester] could exert biopesticide potential [65]. However, these isolated compounds should be further evaluated separately for their potential application in agriculture as a biopesticide. Taken together with these reports, Roiba and Stevenson [66] have strongly recommended *A. conyzoides* as a potential biopesticide, which is very beneficial on insects including ladybirds, spiders, and hoverflies.

Further, allelochemicals can restrain growth of other weeds; however, they are not much effective to themselves or the weeds from the same families. The allelopathic activity was evaluated in *Sesamum indicum* L. using aqueous ACE at different concentrations (5, 10, 15, and 20%). The ACE was inhibitory to seed germination, shoot and root development of sesame plants. There was an increase in allelopathic effect with a gradual increase in ACE concentrations [67]. Similarly, the aqueous acetone shoot ACE at different concentrations (0, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/cm³) resulted in allelopathic activity and inhibited the seed germination, shoot and root growth of *Amaranthus caudatus* L., *Lactuca sativa* L. and *Digitaria sanguinalis* (L.) Scop. [68]. Overall, these investigations have potentially indicated that such chemicals of *A. conyzoides* can be effectively utilized for various purposes.

Evaluation of Traditional Medicinal Uses by Molecular Tools

Plants provide many different types of products. From the days of ancient civilizations, plants are widely used as raw materials in different types of medicine [69]. This knowledge of traditional remedies is extensively utilized for new drug development by the contemporary industry [70]. Many

sophisticated biotechnological interventions viz., tissue-culture, marker-assisted breeding, DNA microarrays, metabolomics, proteomics, functional genomics, bioanalytics, etc., are used nowadays in novel drug discovery and formulations from ethnomedicinal plants [71]. *A. conyzoides* has been traditionally used as a medicine by local indigenous people from the majority of globe to cure various diseases/ailments (Table 1). The plant is utilized to cure dyspnea, skin diseases, ulcer, wound, etc., and also as a purgative, and febrifuge. In some African countries, the plant is useful in conventional medicine due to its anti-asthmatic, anti-spasmodic, and hemostatic properties [72, 73]. The ACE contains numerous phytochemical compounds that may have different therapeutic activities such as antioxidant, antibacterial, antimicrobial, anticancer, etc., either from crude or tissue-specific fractions (Table 2) [8, 9, 74]. It has been also used to cure urinary and prostate-related issues in traditional practices for a long time. However, there is a deficit of gene expression studies and evaluation of the pharmacological activity of *A. conyzoides* using advanced molecular tools. Pharmaceutical treatments of prostate swelling (Benign prostate hypertrophy- BPH) include the use of 5- α -reductase inhibitors, which loosen up the muscles in the region of the urethra and bladder to mitigate urine retention. The effect of ACE on the gene expression of 5- α -reductase was assessed for efficacy and safety in treating BPH. The extract showed a significantly reduced human 5- α -reductase mRNA level in prostate epithelial cells. Thus, *A. conyzoides* could potentially be used for the treatment of BPH by decreasing the enzyme activity of 5- α -reductase [74], and can be employed after purifying the active component from ACE with subsequent clinical studies.

Progress in bioinformatics has enormously facilitated the identification of target drug molecules and their interactions using computational algorithms before they can be used for further experiments. In silico analysis not only helps to screen and design potential drug targets for various diseases using isolated phytochemicals but also to identify and predict the metabolic fate of such compounds. Phytochemicals isolated from ACE have been virtually screened using in silico studies for their potential application in biopharma. Several compounds viz. precocene I, β -sitosterol, precocene II, 6-vinyl-7-methoxy-2,2-dimethyl chromene (VMDC), stigmasterol, polymethoxyflavone, pyrrolizidine, neophytadiene, phytol, and caryophyllene isolated from ACE have been used to screen their potential application against various drug targets (e.g., MMP-9, p53, cox-2, α -amylase, etc.) for various diseases like malaria, diabetes, breast cancer and cervical cancer [75–78]. Recently, key secondary metabolites (e.g., Kaempferol, Quercetin, etc.) isolated from ACE have shown potential as drug candidates against the SARS-CoV-2 virus. In silico molecular docking analysis has indicated that these metabolites may inhibit the replication of the

virus by interacting with the active site residues of the main protease enzymes [79]. Thus, virtual screening and molecular dynamic simulations can be crucial tools to design and identify target drug candidates against various diseases in the future.

Numerous pro- and anti-inflammatory mediators are produced during the inflammatory process. From the leaves of *A. conyzoides*, eupalestin, 5'-methoxy nobiletin (MeONOB), and 1, 2-benzopyrone were isolated and evaluated for anti-inflammatory effects. Various cytokines, genes, and enzymes involved in inflammation response were also analyzed [79, 80]. Isolated compounds and other fractions of ACE significantly reduced ($p < 0.05$) the levels of myeloperoxidase, nitric oxide, adenosine deaminase, leukocyte influx, etc., and also reduced the p-p38 MAPK and p-p65 NF- κ B levels. The isolated compounds might have prevented the activation of MAPK and NF- κ B; and thus, attributing to the anti-inflammatory response of *A. conyzoides* [80]. Restricted movement with pain in joints due to inflammation is the main feature of Osteoarthritis (OA) [81]. The effect of leaf ACE on the OA has been studied in rats that might suggest how TNF- α and MMP-9 affect the proteoglycan swelling and degradation during the OA. The leaf ACE (at 160 mg/ 200 g body weight) significantly lowered the MMP-9 and TNF- α level. This suggested that ACE can reduce cartilage inflammation and degradation by potentially inhibiting the activities of TNF- α and MMP-9, respectively [82]. Also, the level of MMP-9 expression is the key indicator of triple-negative breast cancer disease diagnosis. Recently, Hariono et al. [78] used the *n*-hexane fraction of ACE to assess its inhibitory potential against MMP-9. The compound oxytetracycline (OTC) isolated from *n*-hexane fraction had a potent inhibitory effect against MMP-9 with IC_{50} value of 246.1 μ g/ mL. Further, molecular docking analysis suggested that OTC inhibited MMP-9 activity by binding to the PEX9 domain rather than the catalytic site. Therefore, due to higher selective inhibition of MMP-9, OTC can be a safe and promising candidate against triple-negative breast cancer [78]. With the tremendous progress in advanced molecular technologies, more focused studies in the future would bring out numerous beneficial uses affirming biomedical properties of *A. conyzoides*.

Tissue-Culture and Secondary Metabolite Production from *A. conyzoides*

Although the Indian subcontinent contains the richest source of ethnomedicinally important plant species with approximately 45,000 plants, many of these resources are rapidly turning extinct [83, 84]. Medicinal and aromatic plants (MAPs) can produce a diverse array of secondary metabolites generating an invaluable resource of plant-derived

bioactive compounds [85]. These are mainly represented as phenolics, nitrogen-containing compounds, terpenes, and terpenoids [86]. Such secondary metabolites from MAPs are widely utilized as a natural cure for various diseases. However, for large-scale production and proper exploration of medicinal properties with contributory secondary metabolites as well as conservation of MAPs, standard cell, organ, and tissue-culture protocols are required. Plant cell, tissue, and organ cultures offer an efficient homogeneous, controlled production of secondary metabolites, especially to meet commercial demands. These techniques have facilitated the identification and de novo synthesis of novel compounds in higher amounts than the intact natural plants [87, 88]. Tissue-culture techniques especially micropropagation can be a promising tool for the mass multiplication, secondary metabolites production, and conservation of these medicinally important plants [89, 90]. However, very scanty information exists on the tissue-culture-related efforts on *A. conyzoides*. Earlier, a micropropagation protocol was developed from nodal explants of *A. conyzoides* [91]. Using different combinations of auxin and cytokinins in Murashige and Skoog (MS) medium the multiple shoot induction was achieved. A maximum no. of shoots/ explants was observed with a combination of indole-3-acetic acid (IAA) (3.0 mg/L) and 6-benzyl aminopurine (BAP) in MS medium. The elongation of multiple shoots was observed with 3.05 mg/L of BAP and IAA along with 600 mg/L activated charcoal containing MS medium. Out of different auxin-cytokinin combinations, IAA-BAP combinations at 2.0 and 3.0 mg/L concentrations exhibited the greatest induction of multiple shoots and roots, respectively. Similarly, Renu and Nidhi [92] also developed in vitro tissue-culture protocol using various mature plant parts of *Adhatoda vasica* Nees and *A. conyzoides* to explore and analyze the sterol content as they are extensively used in traditional medicine. β -sitosterol is the active phytosterol constituent present in the root, stems, and leaves of both of these plants. In *A. vasica*, callus culture from nodal explants was developed on MS medium containing BAP and α -naphthalene acetic acid (NAA) at 0.5 mg/L and 2.5 mg/L, respectively, whereas for *A. conyzoides*, stock callus was developed from nodal segments on MS medium with 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and BAP at 3.0 mg/L and 0.5 mg/L concentrations, respectively. The callus culture was maintained for 18 months by frequent sub-culturing and analyzed for sterol contents. TLC and infrared (IR) spectroscopy confirmed the stigmasterol and β -sitosterol presence in different plant parts of both species. The six-week-old tissue of *A. vasica* exhibited higher concentrations of total sterol [0.439 mg/gram dry weight (g d.wt.)], β -sitosterol (0.272 mg/g d.wt.) and stigmasterol (0.167 mg/g d.wt.) compared to *A. conyzoides* tissue (total sterol—0.432, β -sitosterol—0.270 and stigmasterol—0.162 mg/g d.wt., respectively). In vivo studies

of different tissues also revealed that *A. vasica* contained slightly higher levels of steroids compared to *A. conyzoides* [92]. In another separate investigation, nodal, leaf, and shoot-tip explants of *A. conyzoides* were utilized for in vitro culture protocol on MS medium having different 2, 4-D, and NAA levels [93]. The leaf explants exhibited the highest (86%) frequency of callus induction when fortified with NAA (at 2.0 mg/L) and 2, 4-D (2.5 mg/L). The auxiliary meristem (67% of explants) produced shoots (2.70 ± 1.05 shoots per explants) when placed on MS medium containing 1.0 mg/L BAP and 0.5 mg/L NAA. Rooting of explants was accomplished with NAA (2.5 mg/L) and indole-3-butyric acid (IBA at 3.0 mg/L). About 86% of nodal explants produced shoots when placed on MS medium containing NAA (1.0 mg/L) and BAP (1.5 mg/L) with 2.16 ± 0.75 shoots per explants. Similarly, MS medium having IBA (3.0 mg/L) and NAA (2.5 mg/L) were used in rooting of explants and this led to 86% response with 6.5 ± 1.04 roots per explants. These were then shifted to small cups filled with sand, vermiculite, and soil (1:1:1 combination) for hardening and later transferred to soil successfully for acclimatization. Additionally, endophytes are a promising source of biologically active compounds having potential applications in the medical, agricultural, and industrial sectors [94]. Endophytic microbes form a symbiotic association with their plant partners. They help plants to absorb nutrients efficiently and also provide protection against biotic and abiotic stresses. There are some reports indicating the presence of endophytes on *A. conyzoides* plants with several endophytic bacteria [94, 95] and fungi [96] that exhibit plant growth-promoting (PGPR) [94] as well as pharmacological activities [95, 97] by producing some important secondary metabolites (e.g., xanthorrhizol, orsellinic acid, *p*-hydroxybenzoic acid, scalariolide, 2-amino-3-quinoline carbonitrile and boric acid and stigmaterol). Overall, tissue-culture protocols can facilitate mass propagation and in vitro germplasm conservation of *A. conyzoides* as well as secondary metabolite production for various utilities [93].

Role of Advanced Molecular Technologies for Better Utility of *A. conyzoides*

In the last few decades, PCR-based tools and other advanced molecular technologies have been effectively used to forecast disease epidemiology as well as for the early detection and characterization of various disease-causing pathogens at different stages of crop development. Begomoviruses have become devastating pathogens for diverse crops due to intensive farming and transportation of plant material globally, as well as recombination and pseudo-recombination (reassortment of genetic materials) of viruses [98]. Several begomoviruses, namely ageratum yellow vein virus (AYVV),

ageratum enation virus, tomato yellow leaf curl Tanzania virus, etc., reside naturally on *A. conyzoides* [99, 100]. The viral proteins collectively capture the host cellular processes hampering/stifling the plant defense cascade. This leads to disease development by inducing programmed cell death and altering host metabolite biosynthesis with leaf enation, crumpling, yellowing, and stunting symptoms in plants [101, 102]. Additionally in opium poppy (*Papaver somniferum* L.), leaf curling with vein thickening was reported through ageratum leaf curl betasatellite and ageratum enation virus. The qRT-PCR analysis from the infected poppy samples suggested significant gene expression variations in the alkaloid pathway. As a result, the content of several key alkaloids such as codeine, morphine, papaverine, etc., was decreased, while the noscapine level was enhanced in the infected plants. Overall, such metabolite variations might lower the market worth of the poppy and other plants [101]. Consequently, begomoviruses infecting crops and other non-crop species can be detected efficiently by circomics (based on rolling circle amplification (RCA), RFLP, and pyro-sequencing combination) [103]. Circomics is a proficient and economic technique to detect geminiviral genomic components including their satellites without any a priori knowledge of their nucleotide sequences [104]. The genomic DNA and target gene sequences from the virus can be utilized effectively for developing virus-resistant crop varieties. Pathogens alter the host plant's defense mechanism and release cell wall degrading enzymes leading to successful disease development [105]. Some viruses also reported hijacking host RNA interference (RNAi) defense pathway that helps to increase their virulence while suppressing and silencing the host genes [106]. Additionally, whitefly (*Bemisia tabaci* Genn.) is a carrier of these begomoviruses that can affect diverse plant species such as crops, weeds, medicinal and aromatic plants [107, 108]. Globally, the whitefly is a serious threat to many important plants due to the high invasiveness of the pest [109, 110]. This pest damages plants in multiple manners, such as (1) by direct infection, (2) by honeydew secretion to attract fungi on the infected sites, and (3) via begomoviruses [111, 112]. Begomoviruses are infecting various plants worldwide due to increased levels of biotypes B and Q of *B. tabaci* [110, 111]. In addition to this, the Q biotype is highly defiant to most insecticides (e.g., pyriproxyfen and neonicotinoid), thus making it difficult to globally manage *B. tabaci* and begomoviruses [113]. For example, normal tomatoes can have begomoviruses (AYVV) natural transmission from the infected *A. conyzoides* via whitefly (*B. tabaci* B biotype) on the Ishigaki Island [114] resulting in severe yellowing and curling of tomato leaves (Fig. 3A). As a protection strategy through the utilization of viral genome sequences, *A. conyzoides* can be engineered with antimicrobial peptides or compounds [115] to directly suppress the virus infection (Fig. 3B). Alternatively, the vector- whitefly can also

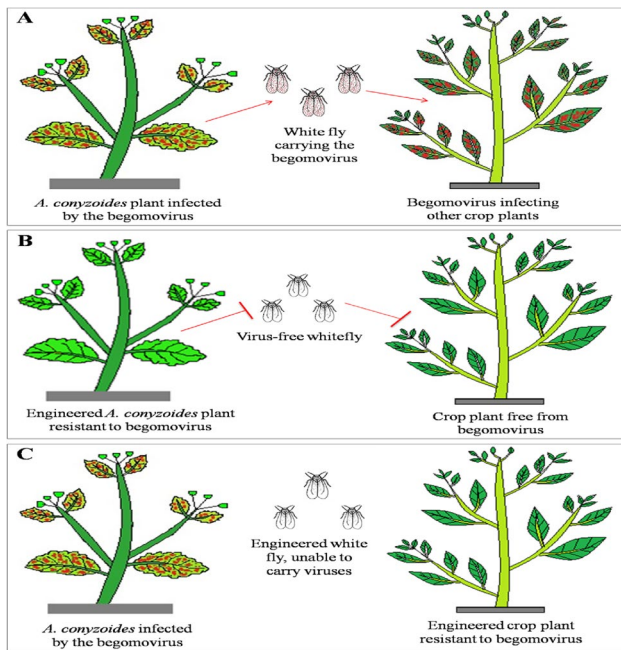


Fig. 3 Transmission of begomovirus from *A. conyzoides* to other crop plants and potential ways to reduce such infection. **A** Begomovirus gets transmitted through whitefly from host to other crops, **B** engineered *A. conyzoides* plant is resistant to begomovirus infection and thereby begomovirus transmission is blocked, **C** engineered crop plant with resistance against begomovirus or engineered whitefly with inability to carry pathogen (virus-free) can offer valuable protection to nearby plants and thus, can minimize the damage from begomovirus

be targeted by advanced genetic engineering tools to make them incapable of carrying these pathogens, and thereby, damage to other crops and surrounding plants can be minimized (Fig. 3C). Further by targeting viral RNA for degradation, the engineered host plant can have strong immunity [116]. In the last few years, the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats /CRISPR-associated 9) genome editing approach has demonstrated a potential to develop virus-resistant plants [117]. CRISPR/Cas9 can offer an antiviral defense strategy, wherein an RNA-directed nuclease (frequently a Cas protein) slices a viral DNA or RNA at specific target sites complementary to CRISPR RNA, leading to their degradation [118]. Thus, based on modern molecular technologies and targeted gene manipulation via genome editing tools can facilitate future development of broad-spectrum resistance.

To develop proper weed management schemes molecular studies would be essential that can greatly help to explore the interplay of genotype-phenotype. Techniques of restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs), and microsatellites have been applied for weed genomics exploration, which may facilitate the selection

of proper biocontrol agents [119]. Additionally, Rowe et al. [120] determined higher genetic variation among populations of *Euphorbia esula* L. from North America through the genetic diversity assessment using RFLP and RAPD. Similarly, various molecular markers are successfully utilized to understand genetic diversity levels among and within weed species from different geographical regions. However, there is a dearth of such studies on *A. conyzoides* till now. With RAPD and ISSR markers, Dieu and Ni [121] have evaluated the genetic diversity of 14 different *A. conyzoides* accessions from different places of the Mekong Delta in Vietnam. Eleven primers (3 RAPD and 8 ISSR) amplified a total of 92 fragments (30- RAPD and 62- ISSR). The percentage of polymorphism and polymorphic bands per primer were 59.78% and 6.11 ± 2.72 , respectively, indicating a greater genetic diversity among *A. conyzoides* plants from different locations. UPGMA dendrogram separated all the accessions into three discrete groups. Thus, a high degree of genetic diversity might indicate that *A. conyzoides* plants are well adapted to environmental changes [121]; however, more such studies would be necessary to make any meaningful outcome.

Herbicides and pesticides have long been used in crop fields to manage weeds and increase agricultural production [122]. Agricultural intensification and higher production requirements have lead to inefficient utilization of these herbicides and pesticides. Subsequently over time, the majority of the weed species become resistant to these chemicals. Detection of point mutations leading to target-site resistance has provided the potential molecular mechanism(s) for herbicide resistance. Lately, next-generation sequencing (NGS) methods are applied for whole-genome sequencing of mitochondria as well as chloroplast from *A. conyzoides* [123, 124]. Additionally, genes governing complex non-target-site resistance for herbicide metabolism and translocation have been detected using NGS [14]. The knowledge from weed genomes will facilitate creating solutions for tackling specific weeds. Epigenetic changes, gene copy number variation, and resulting altered gene expression are potential reasons for herbicide tolerance in many weed plants [125, 126]. Bioinformatics, genetic and genomic studies are essential to understand weed adaptation mechanisms under changing environmental and management conditions. Global food security is facing major trouble due to the yield reduction in several crops although major advancements are being made in breeding, genetics, and other technologies [127]. The major reason behind reduced crop yield is the loss of genetic diversity (genetic erosion) due to the selection of uniform crop varieties with specific traits for a particular environment as they adapt to local conditions [128]. Wild relatives and agricultural weed of crops can provide better resources of genetic diversity that can be utilized for crop improvement [127]. Many weed plants have a strong tolerance against

Table 1 Details of country- and region-wise various uses of *A. conyzoides* with different disease/ailment and treatments

Country	State/Region	Ailments/diseases treated	Tissue used	Mode of preparation	Mode of utilization	References code no. #
Angola	Uíge, Northern Angola	Fever, Eye disease, childhood disease (growth disorders), abdominal pain	Whole plant, leaf	Leaf sap from crushed leaves, with <i>Ocimum gratissimum</i> and <i>Dysphania ambrosioides</i>	Decoction	1
Bangladesh	Chittagong Hill Tracts	Treatment of Snakebite	Leaves	Leaves are crushed to extract paste	Topical	2
China	Jianghua County	Heat clearing and detoxifying, diminishing inflammation, stopping bleeding	Whole plant	-	External uses	3
Ecuador	San Lucas Parish, Southern Ecuador	Gangrene and infection	Whole plant	Plant is crushed and taken orally	Oral	4
Ghana	Ejisu-Juaben Municipality, Southern Ghana Greater Accra and Brong-Ahafo regions	Eye disease, constipation Mental and neurological disorders	Leaves, roots Leaves	Rub and squeeze, Grind The fresh leaves are macerated and the liquid obtained is instilled into the nostrils; the fresh leaves can also be boiled, sieved and drank as required	Topical, Enema Oral, decoction and topical	5 6
India	Uttarakhand	Wound healing	Leaves	The leaf juice is applied topically on cuts to stop bleeding. The whole plant decoction is taken orally to cure leprosy	Topical/oral, decoction. The leaf juice is applied externally on cuts to stop bleeding	7
	Cooch Behar district of West Bengal	Burns, cuts and wounds, skin disease Cuts and wounds	Aerial parts Leaves	Aerial plant parts extract and paste applied Leaves crushed to extract juice and pastes	Applied directly on burns, cuts and wounds Leaf juice is given to cure bleeding from cuts and wounds. Plant paste is applied to cure muddy wounds between toes during rainy season	8 9
	Tripura	Cuts and wounds	Leaves	Leaves are crushed to extract paste	Leaf paste is applied on cut and wounds	10
	Northern Bengal	Cuts and wounds	Leaves	Leaves are washed and crushed for extracting its juice	Leaves juice is applied on cuts including leaves. It is tied with a piece of cloth, which results in healing cuts as well as stops bleeding	11

Table 1 (continued)

Country	State/Region	Ailments/diseases treated	Tissue used	Mode of preparation	Mode of utilization	References code no.*
Nepal	Macchegaun	Cuts and wounds	Shoots	Shoots are crushed for juice and paste preparation	Fresh shoot juice is applied on the cuts and wounds for rapid healing; plant decoction mixed with pepper paste is given in acute stomachache	12
	Darchula District	Cuts and wounds	Leaves	Leaves crushed to extract the juice	Applied on skin to treat cuts and wounds	13
Nigeria	Ebem-Ohafia District, Abia State	Treatment of wound, ulcer, sleeping sickness, eyewash	Whole plant	The plant is macerated to prepare paste	Paste applied to affected area	14
Pakistan	Chenab riverine area, Punjab, Pakistan	Jaundice, wounds, febricity, cough, flu, sexual dysfunction, hair fall, cataract, indigestion	Leaves, roots, stem, flowers, whole plant	Paste, juice, powder extract are prepared by crushing different plant parts	Topical, oral and as eye drop	15
	Hafizabad district, Punjab, Pakistan	Cut and wounds, fever, cold and cough, infertility, jaundice, hair tonic, conjunctivitis, stomachache	Leaves, roots, stem, flowers, whole plant	Different plant parts are crushed to extract juice, paste and powder	Oral, topical and as eye drop	16

*References cited above are given in the supplementary file (suppl. table S1) corresponding to the number presented here

Table 2 Details of various pharmacological activities tested from different tissues of *A. conyzoides*

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
Antibacterial activity	Leaves	Petroleum ether, chloroform, acetone and methanol	Agar well diffusion assay	Gram positive: <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> Gram negative: <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> and <i>P. aeruginosa</i>	Petroleum ether and acetone extract exhibited highest activity against all tested bacteria. Chloroform and methanol extract showed moderate activity	1
	Whole plant	Essential Oils (EOs)	Agar disk diffusion	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , followed by <i>S. mitis</i> , <i>Micrococcus luteus</i> , <i>P. putida</i> , <i>P. desmolyticum</i> and <i>Candida albicans</i>	The strongest antibacterial activity was observed against <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i>	2
	Whole plant	Aqueous, methanolic extracts and their fractions	Kirby-Bauer disk diffusion susceptibility test	<i>Helicobacter pylori</i>	The hexane: ethyl acetate (20:80) fraction exhibited highest antibacterial activity. The recorded MIC and MBC were in range of 0.002–0.500 mg/mL and 0.016 – 1.000 mg/mL, respectively	3
Leaves and stem	Leaves and stem	Essential oils	Disk diffusion method	Gram Positive: <i>Enterococcus faecalis</i> , <i>S. aureus</i> Gram negative: <i>Klebsiella pneumoniae</i> , <i>Shigella</i> spp.,	EOs showed moderate activity against <i>S. aureus</i> and <i>E. faecalis</i> . Very low activity was observed against other bacteria. The inhibition zones and MIC were in the range of 6.7–12.7 mm and 64–256 µg/mL, respectively	4
				<i>S. epidermidis</i> and <i>Propionibacterium acnes</i>	The extract exhibited antibacterial activity with a MIC value of 2.5% against both organisms	5
Leaves	Ethanol extract	Disk diffusion method				

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
	Leaves	Ethanol extract	Disk diffusion method	<i>S. aureus</i> , <i>S. epidermidis</i> and <i>Propionibacterium acnes</i>	The extract showed greater antibacterial activity with MIC with 5 mg/mL, and 10 mg/mL for <i>S. aureus</i> , and <i>P. acnes</i> , respectively, while 7.5 mg/mL for <i>S. epidermidis</i>	6
	Leaves	Methanol extract	Agar gel diffusion method	<i>E. coli</i> , <i>S. aureus</i> , <i>Streptococcus pyogenes</i> , <i>Salmonella enterica</i> and <i>Pseudomonas putida</i>	The extract exhibited highest antibacterial activity against <i>S. aureus</i> with 18 mm zone of inhibition, while the least inhibition was observed for <i>S. pyogenes</i> with 10 mm zone of inhibition	7
	Leaves	Crude extract	Agar well diffusion method; broth dilution method	<i>Proteus vulgaris</i> , <i>Stenotrophomonas maltophilia</i> , <i>Hefia alvei</i> , <i>Proteus mirabilis</i> , <i>Serratia marcescens</i> , <i>Enterobacter agglomerans</i> , <i>Citrobacter freundii</i> , <i>Proteus luminescence</i> , <i>Salmonella subtilis</i> , <i>S. rubidaea</i> and <i>Enterobacter gergoviae</i>	<i>Serratia marcescens</i> , <i>Proteus vulgaris</i> , <i>Enterobacter agglomerans</i> and <i>Proteus mirabilis</i> had highest antibacterial effect with zone of inhibition diameter ranging from 15 to 22 mm. In broth dilution method, the MIC of the extract ranged from 25–100 mg/mL	8
Antifungal activity	Flowers, leaves and roots	Methanolic crude extracts		<i>Puccinia arachidis</i>	The extracts exhibited highest activity at 2.5% to 5% levels	9
	Leaves and inflorescence	Aqueous and ethanol extracts	In vitro and in vivo	<i>Aspergillus fumigatus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>A. tamarii</i> and <i>A. ustus</i>	Maximum activity was observed with aqueous extract against <i>A. niger</i> and <i>A. ustus</i> (average 20 mm inhibition zone for each), while the least activity was against <i>A. fumigatus</i> with 7 mm zones of inhibition	10

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
	Inflorescence, leaf, stem and root	Aqueous, Methanol and <i>n</i> -hexane extracts		<i>Fusarium solani</i>	The <i>n</i> -hexane extracts of leaf and inflorescence showed significant growth reduction (84%) in <i>F. solani</i> followed by stem and root extracts having 80% and 72% growth reduction, respectively	11
Antioxidant activity	Leaves	Methanol extract	DPPH scavenging activity; Ferric reducing power assay	In vitro	The extract exhibited 213.57 µg/mL IC ₅₀ value compared to the standard ascorbic acid with IC ₅₀ value of 6.82 µg/ mL	7
	Whole plant	Essential Oils, Ethanol extract	Hydroxyl radical scavenging; Ferric reducing antioxidant power (FRAP); DPPH assay; Benzoic acid hydroxylation method	In vitro	The extract showed the highest antioxidant activity in FRAP and DPPH assay with IC ₅₀ value of 4.48 ± 0.12 and 22.50 ± 3.18 µg/mL, respectively	2
	Leaves	Aqueous, methanol extracts	DPPH assay, Hydrogen peroxide scavenging activity	In vitro	Methanol extract had greater scavenging power with IC ₅₀ of 94.21 µg/mL in pancreas and 75.95 µg/mL in penile tissue compared to aqueous extract	12
Antioxidant activity	Leaves	Methanol extract	DPPH assay, hydrogen peroxide scavenging, inhibition of formation of lipid peroxides, FRAP and Trolox Equivalent Antioxidant Capacity (TEAC)	In vitro	DPPH, hydrogen peroxide and lipid peroxidation assays showed antioxidant activity with the IC ₅₀ values of 48.34 ± 5.38, 85.44 ± 4.53, and 64.23 ± 8.22 µg/mL, respectively	13
	Whole plant; leaf, stem and flower	Aqueous extract	DPPH assay, Folin-Ciocalteu and Glutathione	In vitro	The leaf extract exhibited highest DPPH scavenging activity with IC ₅₀ value of 0.091 ± 0.024 mg/mL and highest total phenol content of 1678.86 ± 40.67 mg/g	14

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
	Leaves	Ethanol extract	DPPH assay, Nitric oxide scavenging activity, Reducing power assay and Ferrous ion chelating ability	In vitro	The extract exhibited significant dose-dependent DPPH and NO scavenging activity with IC ₅₀ value of 18.91 µg/mL and 41.81 µg/mL, respectively compared to the standard ascorbic acid (IC ₅₀ value of 2.937 µg/mL for DPPH and 37.93 µg/mL for NO, respectively)	15
Hypoglycemic activity	Leaves	Aqueous extract		Rats	The extract showed weak activity (39.1% reduction in blood sugar) compared to the standard hypoglycemic drug (69.2% reduction in blood sugar)	16
	Leaf, stem and root	Methanol extract	Antihyperglycemic and hypolipidemic activities	Rats	The leaf, stem and roots extracts significantly reduced fasting blood glucose (FBG) levels in diabetic rats with 38.71 ± 19.41%, 25.64 ± 20.53%, and 34.76 ± 18.03% respectively, compared to the control rats (11.33 ± 8.91%)	17
	Shoots	Ethanol extract	Alloxan-induced diabetic rats	Rats	The extract showed significant hypoglycemic effect on diabetic rats and lowered the blood sugar level from 590.4 to 42.4 mg/dL	18
Schistosomicidal activity	Leaves	Essential oils	In vitro assay	<i>Schistosoma mansoni</i>	EOs exhibited no synergistic effects	19
Cytotoxicity and anticancer properties	Whole plant; Leaf, stem and flower	Aqueous extract	MTT assay	Jurkat, LNCap, MCF7 and PNT2 cell lines	The aqueous leaf extract exhibited weak cytotoxicity against Jurkat cell line with IC ₅₀ value of 408.15 ± 23.25 µg/mL and no activity against other cell lines	14

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
	Leaves	Ethanol, petroleum ether, ethylacetate, and <i>n</i> -butanol extracts	Sulforhodamine B (SRB) assay	Human non-small cell lung carcinoma (A-549), human colon adenocarcinoma (HT-29), human gastric carcinoma (SGC-7901), human glioma (U-251), human breast carcinoma (MDA-MB-231), human prostate carcinoma (DU-145), human hepatic carcinoma (BEL- 7402), and mouse leukemia (P-388) cancer cell lines	The ethanol extract exhibited an IC ₅₀ value of 1.73 µg/mL against P-388 cell line, while the petroleum ether extract had IC ₅₀ values of 14.06, 13.77, and 0.71 µg/ mL against A-549, SGC-7901, and P-388 cells, respectively	20
	Leaves	Ethanol extract	In vitro cytotoxicity assay	HeLa cell line	Ethanol extract of leaves shown weaker cytotoxicity against HeLa cell lines with LC ₅₀ value of 855 µg/mL	21
	Stem	Methanol extract	In vivo Brine shrimp lethality bioassay	Brine shrimp nauplii eggs (<i>Artemia salina</i>)	The extract exhibited greater cytotoxicity with LC ₅₀ value 1.32 µg/mL, compared to the Vincristine sulfate standard (with LC ₅₀ value of 0.689 µg/mL)	22
Antidiabetic effects	Leaves	Aqueous extract	In vivo assay	Rabbits	The aqueous extract at 200 mg/mL showed significant hypoglycemic effect compared to a standard antidiabetic drug-Glibenclamide (0.25 mg/mL)	23
Anti-inflammatory activity	Leaves	Crude extract	Topical acute edema, Rat paw edema test and IL-6 assay	Adult male rats and Swiss albino mice	The glycosidal flavonoids fraction of crude extract exhibited greater anti-inflammatory activity	24

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
	Leaves	Ethanol extract	Rat paw edema test	Sprague–Dawley rats	The leaves extract at 80 mg/200 g B.W. and 160 mg/ 200 g B.W. could decrease the edema volume, increase the area and thickness of articular cartilage, and increase proteoglycan level	25
	Leaves	Standardized extract of poly-methoxyflavones (SEPAc)	Acute nocifensive behavior of mice	Swiss mice	The SEPAc extract exhibited greater anti-inflammatory activity and have the potential to be used to treat pain and inflammation	26
	Leaves	Hydroalcohol	Rat articular incapacitation, Hind paw edema, Cutaneous vascular permeability and In vivo Cg-induced neutrophil migration	Wistar rats	Water soluble fraction of leaves extract inhibited the carrageenin (400 mg/paw) induced edema, but failed to modify the edema induced by dextran (100 mg/paw)	27
Hemostatic effects	Leaves	Aqueous, ethanol extract	Bleeding time, Clotting time	Albino mice	The aqueous and ethanolic extracts showed positive hemostatic effect	28, 29
Anti-ulcerogenic activity	Leaves	Crude powder	Peptic ulcer models	Rats	Leaves exhibited the maximum anti-peptic ulcer activity against ethanol induced gastric ulcers and cysteamine induced duodenal ulcers in rats	30
	Whole plant	Ethanol extract	In vivo and in vitro assay	Rats	Animals treated with 250 and 500 mg/Kg aqueous leaves extract on cimetidine reduced the formation of gastric lesions compared to control group	31

Table 2 (continued)

Activity	Tissue/ material	Compound/ Extract/ Solvent	Method(s) used	Test Organism/Assay type	Results/ Observation	Reference code no.*
Anti-protozoal activity	Aerial parts	Dichloromethane, Chromene, Flavonoids	Anti-protozoal and Cytotoxicity	<i>Trypanosoma cruzi</i>	The crude extract showed significant anti-protozoal activity. The chromene from extract was inactive, while the flavonoids exhibited lower activity against the protozoan pathogens	32
	Whole plant	Eneccalol angelate, eneccalol methyl ether, chromenes eneccalin, and eneccalol	Anti-trypanosomal activity	<i>Trypanosoma brucei rhodesiense</i> , <i>T. cruzi</i> , <i>Leishmania donovani</i> and <i>Plasmodium falciparum</i>	None of the extract exhibited significant anti-trypanosomal activity	33
Anti-malarial Activity	Leaf extract	Aqueous, Chloroquine and Artesunate	Suppressive test and Rane's curative test	Albino mice	Suppressive test showed significant dose-dependent reduction in parasitemia level produced by the extract-chloroquine and extract-artesunate combination. Curative tests showed absolute survival in two extract-drug combination	34
Analgesic activity	Whole plant	Crude extract	Analgesic potential of acetic acid induced writhing in mice	Albino mice	The extract (500 mg/Kg level) showed highest analgesic effect compared to the standard Diclofenac sodium	35
Wound healing potential	Leaves	Aqueous, ethanol extracts	Wound healing efficacy on rats		Topical application of extracts accelerates the rate of wound healing. The tensile strength of the treated tissue was increased by 40%	36

*References cited above are given in the supplementary file (suppl. table S2) corresponding to the number presented here

different abiotic stresses [129]. Identification of such target gene(s) from weeds may offer ways to improve abiotic stress tolerance in crops [130]. These genes can be incorporated into new crop varieties utilizing transgenic approaches and multi-omics techniques [14]. Overall, such new lines/cultivars, thus developed will have improved yield, biomass generation and durable resilience against various biotic and abiotic stresses associated with changing climate and environments [130].

Conclusion

Although considered invasive, *A. conyzoides* L. is globally utilized in traditional medicine to treat different ailments and diseases. This is due to the presence of diverse pharmacological properties. However, the mechanistic basis of such activities from this plant has not yet been properly evaluated through various molecular technologies. Also, there is very scanty information on specific adverse side effects of ACE and thus, calls for such independent research efforts for better utilization of this herbal formulation in biopharma. Additionally, it is very rich in sterols, flavonoids, saponins, chromenes, pyrrolizidine, alkaloids, coumarin, pyrrolone, terpenoids, and lignin. As their biosynthetic pathways with gene regulatory components are not properly deciphered till now, future research exploration in such a direction would be widely useful. Besides, in silico analysis/ computational simulations such as molecular docking, and in vitro/ in vivo studies of such compounds may aid to develop various potential drugs against important human diseases. Further, secondary metabolites of *A. conyzoides* have exhibited greater potential against various pathogens and thus, these could be further investigated to generate environment-friendly biopesticide for green agriculture and a safe environment. Similarly, tissue-culture-based protocols may be utilized in mass propagation and for the generation of important specialized metabolites with modern genomics and multi-omics tools. Moreover, *A. conyzoides* can facilitate the removal of excess nitrogen, phosphorus, and heavy metals from waste or polluted sites. This could be explored further using the nanotechnology-based green synthesis of nanoparticles from plant material that may have various applications not only in agriculture to improve seed germination, growth, and protection of plants to abiotic and biotic stress but also in the removal of heavy metals from polluted areas for a clean environment. The plant is a natural reservoir host of several begomoviruses that transmit the disease to adjacent crop plants. Using advanced molecular technologies, the enhanced knowledge regarding the transfer of the virus from the host to other plants and disease

establishment may facilitate the improved defense in crop plants while safeguarding the environment.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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