PHYTO-PHARMACOLOGICAL PROFILE OF *AGERATUM CONYZOIDES*

Jinal D. Solanki[1*], Khodidas D. Raiyani[1], Biren N. Shah[1], Dikshit C. Modi[1], Dipanshu Shahu[2].

[1]Department of Pharmacognosy, Vidybharti Trust College of Pharmacy, Umrakh, Gujarat, India.
[2]Department of pharmaceutics, Vidybharti Trust College of Pharmacy, Umrakh, Gujarat, India.

* For Correspondence
Jinal D. Solanki
Solankijinal87@yahoo.in
Mob: +918866501950

Summary

Ageratum conyzoides belongs to the family Asteraceae is very common in West Africa and some parts of Asia and South America.. Phytochemical studies show that yield of oil from the petroleum ether extract of the seed was 26 %. most common chemical component of the essential oil of *A. conyzoides* is Chromene, chromone, benzofuran and coumarin. It has medicinal plant properties like neuromuscular blocking activity, calcium blocking activity, analgesic activity, antimicrobial, anticonvulsant and treatment of chronic pain in osteoarthrotic patients. However, it's limited for external use due to toxicity issues. It is also an insecticide and nematicide. In this review we have reported the phytochemical and pharmacological application of the plant.

Key words :- *Ageratum conyzoides,* Insecticidal, phytotoxin, Toxicological application.

Introduction

Ageratum is derived from the Greek words '*a geras'*, meaning non-aging, referring to the longevity of the whole plant. *Conyzoides* on the other hand is derived from '*konyz'* the Greek name of *Inula helenium* which the plant resembles.[1] *Ageratum conyzoides* belongs to the family Asteraceae tribe Eupatoriae. This family is well marked in their characteristics and cannot be confused with any other. A large majority of the plants in the family are herbaceous while trees and shrubs are comparatively rare. The genus *Ageratum* consists of approximately 30 species but only a few species have been phytochemically investigated.[2]

A. conyzoides is a tropical plant that is very common in West Africa and some parts of Asia and South America. It is an annual branching herb which grows to approximately 1 m in height. The stems and leaves are covered with fine white hairs, the leaves are ovate and up to 7.5 cm long. The flowers are purple to white, less than 6 mm across and arranged in close terminal inflorescences. The fruits are achene and are easily dispersed while the seeds are photoblastic and often lost within 12 months.[3] The plant grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites. It has a peculiar odor likened in Australia to that of a male goat and hence its name 'goat weed' or 'billy goat weed'.

The toxicity of this plant has not been well studied however, the essential oil obtained by steam distillation has been reported to have a powerful nauseating odor.[4] The plant has also been found to be poisonous to rabbits due to the presence of HCN and coumarin.[5] *A. conyzoides* is not eaten by humans except when taken for medicinal purposes, but in some cultures it is a delicacy for domestic guinea-pigs, horses and cattle[6]. It is also used to feed fish.[7]

Figure: Ageratum conyzoides

Phytochemical Studies

A large percentage of the publications on the phytochemistry have to do with the essential oil of this plant. The oil content varies randomly from 0.11 to 0.58 % for leaves and from 0.03 to 0.18 % for the roots depending on times of the year.[8] From water distillation of the fresh flowers, the oil content was found to be 0.2 %.[4] The yield of oil from the petroleum ether extract of the seed was 26 %.[9]

Mono- and sesquiterpenes

A large number of constituents have been identified from the GC-MS analysis of the essential oil of *A. conyzoides*. The largest so far, a total of 51 constituents have been reported from the analysis of an oil sample of the plant collected from a university environment in Nigeria.[10] The constituents identified include 20 monoterpenes (6.4 %) and 20 sequiterpenes (5.1 %). The mono- and the sesquiterpenes are obtained in minute quantities (trace-0.1 %). The monoterpenes obtained in approximately 1 % of the oil include sabinene and β-pinene, 1.6 %, β-phellandrene, 1,8-cineole and limonene, 2.9 %, terpinen-4-ol, 0.6 %, and α-

terpineol, 0.5 %. Ocimene which is found in trace amount in the oil from the Nigerian plant, is found to be 5.3 % of the oil from the plant collected in India[11]. α-Pinene 6.6 %, eugenol 4.4 % and methyleugenol 1.8 % are also obtained from the Indian plant oil. The major sesquiterpenes are β-caryophyllene, 1.9 %[10], 10.5% from the oil obtained from Cameroon[12] and 14-17 % in Pakistani oil.[13] δ-Cadinene is another sesquiterpene which has been reported to occur in approximately 4.3% of the oil from Indian plants.[11] Sesquiphellandrene and caryophyllene epoxide have also been obtained in 1.2 and 0.5 percentages, respectively.[10]

Chromene, chromone, benzofuran and coumarin

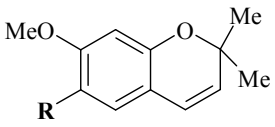
The most common component of the essential oil of *A. conyzoides* is 7-methoxy-2,2-dimethylchromene (precocene I) **[1].** This compound has been obtained in percentages ranging from 30 from Vietnamese oil[14] to 93 from Congo oil.[8] The 6,7-dimethoxy derivative, ageratochromene (precocene II) **[2]** has been found in ranges from 0.7% (25) to 55%[14]. Ageratochromene dimer **[19]**[15, 16] have also been reported from the essential oil. Other related compounds obtained from the oil include encecalin **[6]**, 6-vinyl-7-methoxy-2,2-dimethylchromene **[7]**, dihydroencecalin **[9]**, dihydrodemethoxyencecalin **[10]**, demethoxyencecalin **[11]**, demethylencecalin **[12]**[10], and 2-(1'-oxo-2'-methylpropyl)-2-methyl-6,7-dimethoxychromene **[14]**.[17] The presence of these acetyl chromenes in *A. conyzoides* is believed to be of chemotaxonomic significance. It indicates that the genus is chemically closer to the *Ageritanae subtribe* as opposed to the Piqueriiae group to which it was previously assigned.[10]

In addition to the chromenes obtained from the oil, seven other chromene derivatives are isolated from hexane extract of the aerial part of the plant. These are 2,2-dimethylchromene-7-*O*-β-glucopyranoside **[13]**[18], 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene **[3]**, 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene **[4]**, 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene **[5]**, 6-angeloyloxy-7-methoxy-2,2-dimethylchromene **[8]** and an inseparable mixture of encecanescins **[20–22]**.[19]

Benzofuran derivatives, 2-(2'-methylethyl)-5,6-dimethoxybenzofuran **[17]**[17], 14-hydroxy-2Hβ,3-dihydroeuparine **[18]**[18] as well as chromone derivatives, 3-(2'-methylpropyl)-2-methyl-6,8-dimethoxychrom-4-one **[15]** and 2-(2'-methylprop-2'-enyl)-2-methyl-6,7-dimethoxychroman-4-one **[16]** have also been reported from the plant[17]. The essential oil of *A. conyzoides* from Brazil has been reported to yield 1.24 % of coumarin in addition to two other unidentified coumarin derivatives.[20]

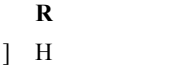
Flavonoids

A. conyzoides is very rich in polyoxygenated flavonoids. To date a total of 21 polyoxygenated flavonoids have been reported from this species only. This includes 14 polymethoxylated flavones **[23-36]**.[21-28, 42] Noteworthy, are the tricin derivatives, 3',4',5'-oxygenated flavones, which are rare in natural product chemistry but occur in good yields in this plant. These examples include 5'-methoxynobiletin **[23]**[22,26], linderoflavone B **[34]**[22], 5,6,7,3',4',5'-hexamethoxyflavone **[25]**[21-23], 5,6,8,3',4',5'-hexamethoxyflavone **[27]**[21], eupalestin **[35]**[21,23] and several others **[28, 30, 32, 33]**.[21,22] Conyzorigun originally believed to be a phenoxychromone[27] was found to be identical with eupalestin.[23] The polyhydroxyflavones include scutellarein-5,6,7,4'-tetrahydroxyflavone[28], quercetin, quercetin-3-rhamnopiranoside **[37]**, kaempferol, kaempferol-3-rhamnopiranoside **[38]**[29] and kaempferol 3,7-diglucopiranoside **[39]**.[29,30] The isoflavone **[40]** obtained from the plant was reported by a group of Indian researchers



R

[1] H
[2] OMe
[3] MeO



[4] HO

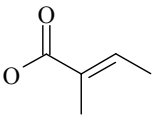
R

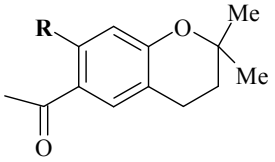
[5] EtO

[6] O

[7]

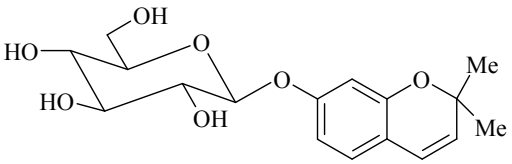
[8]



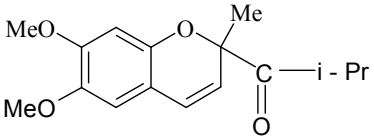


R

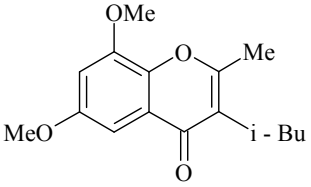
[9] OMe
[10] H
[11] H, Δ^3
[12] OH, Δ^3



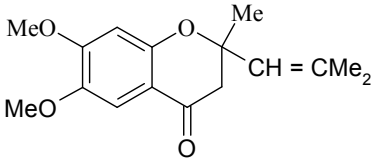
[13]



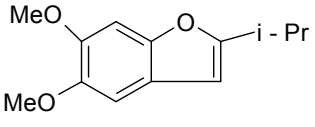
[14]



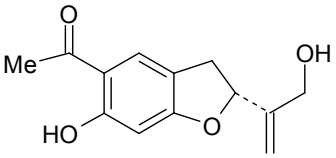
[15]



[16]



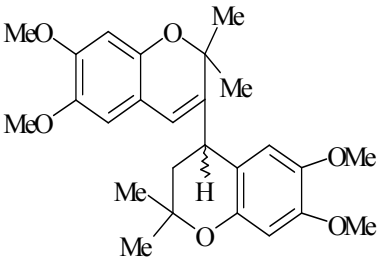
[17]



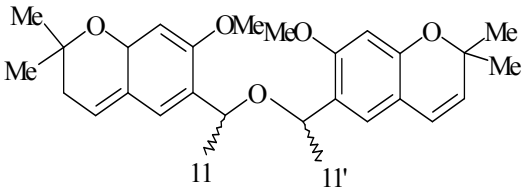
[18]

Chromene, Chromone, Chromanone and Benzofuran.

Chromene Dimer



[19]

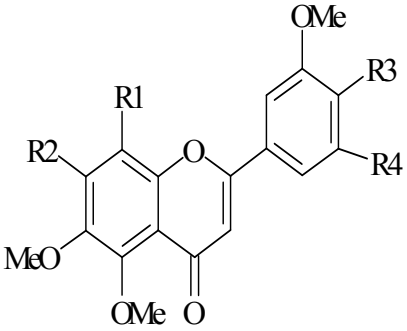


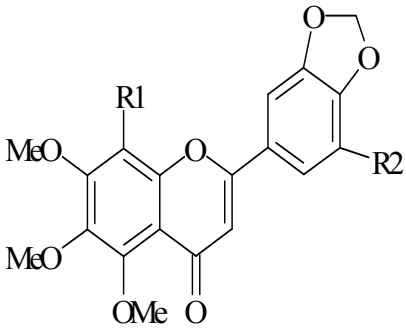
[20] 11a - Me 11' b - Me

[21] 11b - Me 11' b - Me

[22] 11b - Me 11' a - Me

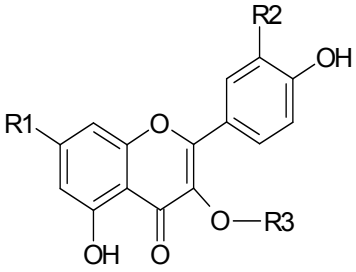
Flavonoids



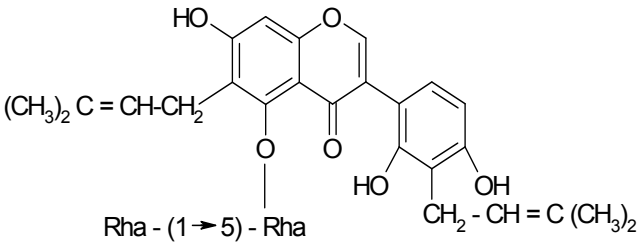


[23] R1 = R2 = R3 = R4 = OMe
[24] R1 = R2 = R3 = OMe, R4 = H
[25] R2 = R3 = R4 = OMe, R1 = H
[26] R1 = R4 = H, R2 = R3 = OMe
[27] R1 = R3 = R4 = OMe, R2 = H
[28] R2 = R3 = R4 = OMe, R1 = H
[29] R1 = R2 = OMe, R3 = OH, R4 = H
[30] R1 = R2 = R4 = OMe, R3 = OH
[31] R1 = R4 = H, R2 = OMe, R3 = OH
[32] R2 = R4 = OMe, R3 = OH, R1 = H

[33] R1 = H, R2 = OMe
[34] R1 = OMe, R2 = H
[35] R1 = R2 = OMe
[36] R1 = R2 = H

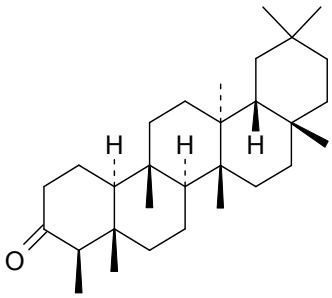


[37] R1 = R2 =OH
R3 = rhamnopyranosyl
[38] R1 = OH, R2 = H
R3 =rhamnopyranosyl
[39] R1 = O-glucopyranosyl,
R2 = H,
R3 = glucopyranosyl

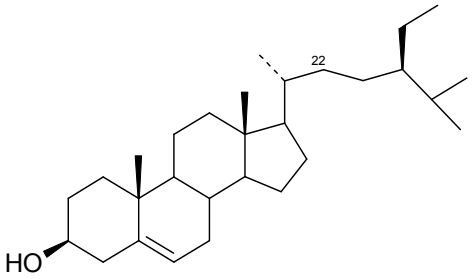


[40]

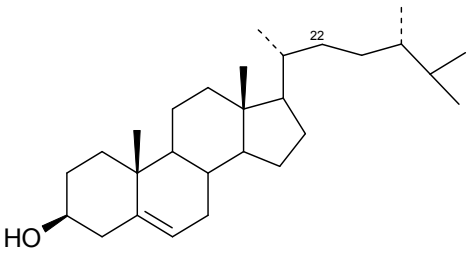
Triterpenoid and Sterols



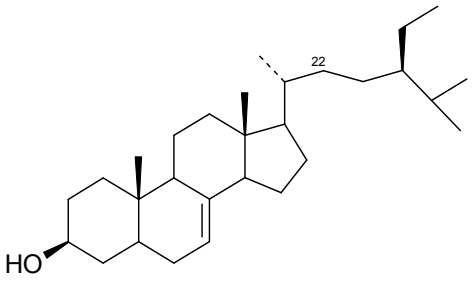
[41] Friedelin



[42] b-Sitosterol
[43] △22 Stigmasterol

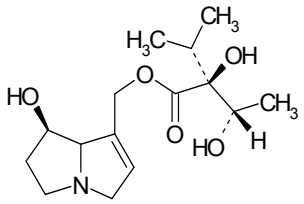


[44] △22 Brassicasterol
[45] Dihydrobrassicasterol

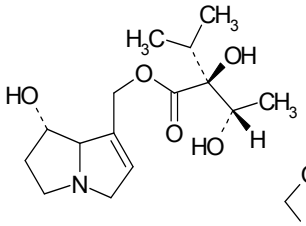


[46] △22 Spinasterol
[47] Dihydrospinasterol

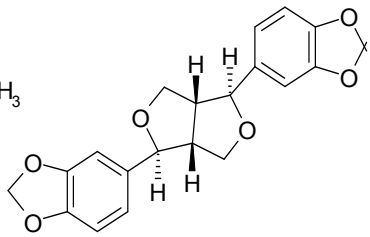
Alkaloids and other Compounds



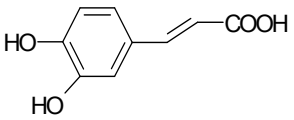
[48]



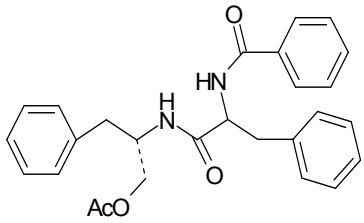
[49]



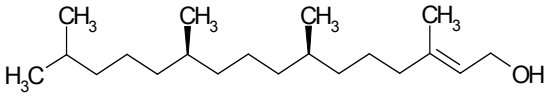
[50]



[51]



[52]



[53]

Triterpene and sterols

The triterpene friedelin **[41]** and the common sterols-β-sitosterol **[42]**, stigmas-terol **[43]** have been isolated from this plant. While the two sterols are major constituents, other minor sterols have also been isolated. These are brassicasterol **[44]** and dihydrobrassicasterol **[45]**, spinasterol **[46]**, dihydrospinasterol **[47]**.[32-34]

Alkaloids and miscellaneous compounds

Lycopsamine **[48]** and echinatine **[49]**, two isomeric pyrrolizidine alkaloids (Pas), are the only alkaloids isolated from this plant. PAs are known to be widely distributed in Asteraceae and in particular in the tribes Senecioneae and Eupatorieae[35].

Other compounds isolated from *A. conyzoides* include (+)-sesamin **[50]**[19], aurantiamide acetate **[52]**[36], fumaric acid,

caffeic acid **[51]**[30], phytol **[53]** and hydrocarbons, from nC_{27}-H_{56} to nC_{32}-H_{66}.[37] (Z)12-6-methyl-heptadecenoic acid was obtained from the essential oil and found to show insecticidal and growth regulatory activity against desert locust *Schistocera gregaria.*[38] The fatty acids composition of the seed[39] and amino acids content of the flowers[40] have been analyzed. Vitamins A and B[41] have also been reported from the flowers.

Biological Studies

The biological activities of the plant are treated under two subheadings: (a). pharmacological and (b) insecticidal and other biological properties.

Pharmacological

Crude extract

The crude extract of the whole plant has been reported to be superior to vaseline gauze as a wound dressing material.[42] It has been found to have neuromuscular blocking activity in isolated rats phrenic nerve-diaphragm and also caused greater fall in diastolic pressure compared with that of systolic pressure in anaesthetized rats. It has calcium blocking activity similar to that of Verapamil.[43] The leaf extract has been used in the treatment of chronic pain in osteoarthrotic patients.[44] Its antimicrobial and anticonvulsant activities have also been demonstrated.[45,46] The methanolic extract of the whole plant also has antimicrobial activity.[47] Aqueous extract of the leaves has been reported to prevent coagulation of the whole blood while causing precipitation of some blood materials. Bleeding time was also decreased in this assay.[48]

The analgesic activity of the leaf extract was detected by hot plate method[49]. The extract decreased spontaneous motor activity and caused a fall in rectal temperature. In vitro receptor radioligand assay was carried out on the extract to demonstrate its selectivity to a single receptor implicated in the mediation of pain.

Results showed that it produced positive results (> 50% inhibition) in the bradykinin (BK II) assay but the activity was lost after PVP treatment suggesting that phenolic compounds could be responsible for the initial bioactivity.[50] The extracts, however, did not produce positive effects in the neurokinin (NK I) and calcitonin gene-related peptide (CGRP) assays.

The water soluble fraction (WSF) of the plant extract contains a peripheral analgesic activity and an anti-inflammatory action, which seems to occur in leukocyte-dependent inflammatory events .[51] It has been shown to be a potent and non-specific blocker of smooth muscle contraction, possessing also a myorelaxing activity.[52] Margort e Silva et al., investigating the effect of the WSF on smooth muscles (using isolated rat uterus and intestine smooth muscles) concluded that the fraction possess substance(s) which provoke direct relaxing effect on smooth muscles and inhibit contraction induced by several agonists possibly by blocking the entry of calcium and/or inhibiting cyclic AMP phosphodiesterase.[53] These pharmacological characteristics could explain the popular use of *A. conyzoides* to alleviate abdominal and menstrual pains.

Clinical trials with patients with arthrosis have been conducted with the aqueous extract of the whole plant. Result shows analgesic effect in 66% of patients and improvement in articulation mobility in 24% without side effect.[54]

Essential oil

Essential oil of *A. conyzoides* has been tested for anti-inflammatory, analgesic and antipyretic activities in mice and rats. At doses of 3 and 4 ml/kg per os, the oil was found to have a significant ($P<0.02$) anti-inflammatory (cotton pellet granuloma) activity. At 3 ml/kg the antipyretic (brewer's yeast injection) effect was comparable with that of reference compound (acetyl salicylate lysine 50 mg/kg per os), whereas the analgesic (tail-flick and writhing test activity) was shown at 2, 3 and 4 ml/kg ($P<0.05$). The daily administration for 7 days failed to show gastric toxicity.[55]

The antimicrobial activity of the oil has been a subject of investigation too.[56-58] Antibacterial and antifungal

activities against 22 bacteria, including Gram-positive cocci and rods and Gram-negative rods and 12 fungi (3 yeast-like and 9 filamentous) showed that the oil inhibited 20 bacteria and four fungi.[56] Of note is the fact that total inhibition of growth was recorded against the four fungi, *Candida albicans* SP-14, *Cryptococcus neoformas* SP-16, *Sclerotium rolfsii* SP-5 and *Trichophyton mentagrophytes* SP-12. Rao[57] reported that the oil inhibited the growth of five bacteria as well as 10 fungi species and that the major component of the oil demethoxyageratochromene **[1]** was effective against two of the fungi, *Penicillium chrysogenum* and *P. javanicum.*[10] The oil provided 100% inhibition of the mycelial growth and germination of spores of *Didymella bryoniae.*[58]

Metabolites

Pharmacological activities of the most significant metabolites, besides the essential oil from this plant, responsible for the medicinal properties have not been identified. There are, however, wide spectrums of pharmacological activities of the classes of compounds obtained from this plant. For example, simple chromenes and chromans especially the 6-amino and 6-acetamido derivatives have been reported to have anti-depressant, analgesic and antipyretic properties. Some of them possess activity against flat worms of the order of trematodes.[59] Other simple 2,2-dimethyl chromene derivatives like 6-(1-hydroxyethyl)-7,8-dimethoxy-2,2-dimethylchromene and 6-hydroxy-7,8-dimethoxy-2,2-dimethylchromene have been shown to have antimicrobial activities.[60]

The sterols, especially stigmasterol, have been shown to exert significant antiinflammatory activity.[61] The flavonoids possess a wide range of biological activities. The list includes effects on central vascular system, diuresis, spasmolytic, antiviral, anti-inflammatory properties.[62,63] The free radical scavenging and anticancer activities of the flavonoids are of public knowledge. Even though the biological activities of the flavonoids isolated from *A. conyzoides* have not been investigated, it is pertinent to note that four polymethoxyflavones isolated from Citrus juices have been shown to be important candidates for cancer-

protective action.[64] Two of these poly-methoxy-flavones are the same as those earlier isolated from *A. conyzoides* **[24, 26]**. They are found to have potent activity for inducing differentiation of human promyelocytic leukemia cells (HLC).[60]

Insecticidal and other biological properties

Insecticidal activity

A. conyzoides has bioactivity that may have agricultural use. The insecticidal activity may in fact be the most important biological activity of this species. Both the essential oil as well as the major components of the oil, namely the precocenes, have been reported to have antijuvenile hormonal activity. The oil exerted acute toxicity on adults of cowpea weevil, *Callosobruchus maculatus* F. upon fumigation. Application of oil dressing on cowpea seed exhibited insecticidal activity against weevil. Significant oviposition deterrence and complete inhibition of emergence of adult insects (F1 offspring) from oil-treated beans were evident at 2.5 to 10 µl/9.5 g beans with no adverse physiological effect. Precocene I was found to be four times as active as the oil.[65]

Assays conducted in India showed high nymphal mortality (91%) of the oil to the Nymphs of *Schistocerca gregaria* [17]. Calle et al. showed that the hexane extract of the whole plant showed activity against *Musca domestica* larvae[66]. Methanolic extract from fresh leaves (250 and 500 ppm) also produced deficiency of juvenile hormone in the fourth instar of *Chilo partellus*, a sorghum pest.[67] Antijuvenile hormonal activity of Precocenes I and II have been demonstrated on a variety of insects which include *Sitophilus oryzae*, *Thlaspida japonica*, *Leptocarsia chinesis*[68] and *Dysdercus flavidus.*[69,70] The results from these assays include precocious metamorphosis of the larvae, production of sterile, moribund and dwarfish adults. The two chromenes have been reported to act synergistically and they survived metabolism for at least 12 days.[69] Preliminary study on the mode of action of precocene II on *Musa domestica* L. and *Lucilia caesar* L. have been carried out.[71] While the

precocenes have been seen as fourth-generation insecticides, the drawback is that they have been shown to cause hepatotoxicity in rats.[72-74] This is an important factor bearing in mind the human health hazard in field applications of precocenes as large-scale insecticidal agents. The mechanism of action has been carried out by a number of researchers. Some workers demonstrated that the toxicity was due to a highly reactive precocene-3,4-epoxide, a metabolite produced in insect species from cytochrome P-450.[72,73] Others, like Darvas and colleagues[75], Casas et al.[76] reported that the 3, 4 double bond played no significant role in the toxicity but that the oxidative dealkylation process at C7 position, as a tocopherol-like antioxidants, might be responsible for the cytotoxicity.

Allelopathy and Allelopathic Potential

Both the volatile oil and the aqueous extract of the *A. conyzoides* have been shown to have allelopathic effects on a number of cultivated crops. These include radish, mungbean and ryegrass.[77] The saturated aqueous solution of the isolated and purified precocene I and II have been reported to have significant inhibitory effect on the seedling growth of radish, tomato and ryegrass.[78] The allelopathic potential of the aqueous extract from different organs of *A. conyzoides* and from its different development stages especially from different habitats, was different.[79]

The inhibitory effects of AC volatiles on peanut, redroot amaranth, cucumber and ryegrass increased when plants were grown under nutrient-deficient conditions or in competition with *B. pilosa*; however, there was no difference with physical damage or 2,4-D treatment. Phytoinhibitory effects decreased under fungal infection and aphid feeding. Volatiles from AC plants infected with *E. cichoracearum* or exposed to *A. gossypii* feeding inhibited or killed fungi and insects.[80]

Phytotoxic Action

A. conyzoides showed strong inhibition on R. sativus, L. germination and growth in a bioassay. The leaves exhibited a greater suppression than the stem and root.

Application of AC leaves at 2 t/ha in a paddy field 2 days after transplanting caused about 75% paddy weed reduction and increase yield by 14% compared with and herbicide treatment. The greater number of growth inhibitors (phenolic compounds) found in the leaves might result in the stronger inhibitory activity than the stem and root. AC might be a natural herbicide for weed control in paddy fields to reduce the dependence on synthetic herbicides.[81]

Toxicological studies

The pollination season of the plant causing allergy has been found to be between November and January in Delhi. In another study, Ageratum (species not mentioned) pollen were reported to be cause of nasobronchial allergy in 5 out of 50 patients.[82]

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