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Qualitative Determination of the Pyrrolizidine Alkaloids in Different Traditional Medicinal Plants

Sharma S[1] and Agrawal R[2*]
[1]*Department of Chemistry, Venketeshwara University- Gajraula, Amroha (U.P.).*
[2]*Department of Chemistry, CCS University Meerut (U.P.).*

Abstract*: Pyrolizidine alkaloids are toxic for human and livestock. Various reports have been investigated the PA's intoxication in human as well as animals. Plants containing hepatotoxic PAs are present in most parts of the world and often cause poisoning in cattles and also in human. Several traditional popular medicines of India have been reported for successful treatment of various diseases. Among these medicines some were found to have Pyrrolizidine alkaloids which showed long term toxicity effect in human. The other source of Pyrrolizidine alkaloids exposure is consumption of grains contaminated with toxic PAs containing plants by human and grazing livestock which enter food chain and cause intoxication. In the present study some reported PAs containing medicinal plant parts were collected and analysed for presence of Pyrrolizidine alkaloids qualitatively and compared for the quantity with each other.*

Keywords: *Pyrrolizidine alkaloids, Traditional Indian Medicine, TLC, Senecio vulgaris, Trichoderma.*

I. Introduction

Pyrolizidine alkaloids comprises a large class of natural products which are secondary metabolites produced by a large number of plant species. The availability of pyrolizidine alkaloids in plants depends on the types of plant species, and part of the plant and also affected by the other physical factor [1]. Pyrrolizidine alkaloids (PAs) are found in some plants of the Apocyanaceae, Asteraceae, Boraginaceae, Compositae (Senecionae and Eupatoriae), Fabaceae, Leguminosae (Crotalaria), Rannuculaceae and Scrophulariaceae families [2]. More than 95% PA containing plants have been found belongs to these four families [3].

Several traditional medicines in India are world popular. Various studies revealed successful treatment by traditional Indian medicine, known as "Ayurveda" system of treatment [4]. Various reports of Materia Medica of Ayurveda [5]; The Ayurvedic Pharmacopoeia of India, Govt. of India 2001; Database on Medicinal Plants used in Ayurveda [6] and Ayurvedic Medicinal Plants [7] investigated some PA containing plants. These are five plants which are listed viz., Cordia myxa contains the non-toxic PA macrophylline, Crotalaria juncea which contains junceine and other toxic PAs from the monocrotalinetype, Eupatorium triplinerve (contains toxic PAs of the lycopsamine-type similar to other Eupatorium species), Onosma bracteatum (contains toxic PAs of the lycopsamine-type similar to other Onosma species) and Vanda roxburghii (contain non-toxic PAs of the laburnine type like acetyllaburnnine isolated from Vanda cristata). Investigation demonstrated that PAs containing plants can be used as medicine due to the lack of acute toxicity and they serve as long term toxicants. Because it takes long period between uptake of toxic compounds and outbreak of toxic symptoms. So the toxic side effects of PAs containing medicinal plant cannot be seen consequently [4].

The pyrrolizidine structure conatains two fused, five-membered rings on sharing a bridgehead nitrogen atom, and form a tertiary alkaloid. The rings contain a hydroxymethylene group at the C-1 position and a hydroxyl group at the C-7 position, forming a necine base. Toxicity is thought to be due to enzymatic conversion of PAs to pyrroles, which act as alkylating agents [2]. Due to necine PAs are hepatotoxic, carcinogenic, genotoxic, teratogenic, and sometimes pneumotoxic.

In the present study some reported PAs containing medicinal plant parts were collected from local botanical garden and other resources and subjected for plant extraction and analysed qualitatively and compared for the quantity with each other. The plants which were taken for experiment have been described for their characteristic PAs content and their therapeutic uses in Table 1.0.

Table 1.0: Plants description used for the studies (source literature)

Plant Species	Family	Indian vernacular name	Organ studied	PAs reported	Therapeutic applications	References
Eupatorium conyzoids	Astereraceae	Ajagandha, Gandhari	Leave	Lycopsamine, echinatine	Treatment of kidney stone, cuts ulcers, wound, diarrhoea	[8]
Chromolaena odoratam	Astereraceae	Ropani	Leave	O^7-angeloylretronecine, O^9-angeloylretronecine, renderine, intermedine	Leave extracted used for cuts, wounds, burn, and indigestion, haemorrhages, skin diseases, edema etc.	[9]

Crotolaria verrucosa	Febaceae	Sonapushpi	Leave	Anacrotine, crotaverine, O^{12}-acetyl crotaverine	Leave extract reduces salivation, scabies, dyspepsia, blood impurities, diarrhoea, dysentery, leprosy	[10,11]
Emilia sochifolia	Asteraceae	Dravanti	Leave	Senkirkine, doronine	Conjuctivitis, tosilitis, bleeding piles, allergy, cuts, ulcers, worm infestations	[12,13]
Eupatorium triplinerve	Asteraceae	Ajapana	Leave	Lycopsamine, rinderine, echinatine, supinine, amabiline	Diuretic, antitumor, cathartic,	[14]
Senecio vulgaris	Asteraceae	groundsel	Leave	Usamarine, spartioidine, senecionine, retrorsine	Diuretic, diaphoretic,dysmenorroea, and bilious pain	[15]
Trichodesma indicum	Borginaceae	Surasa	Leave	Trichodesmine	Arthralgia, inflammations, dyspepsia, diuretic, febrifuge, sores and fever, astringent, antidysenteric,	[16]
Rotula aquatica	Borginaceae	Asmabheda	Leave	Ehretinine	Against haemorrhoids, diabetes, venereal diseases	[17]
Andrographis paniculata	Acanthaceae	Kalmegh, kirayat	Leave	PA not present others Andrographolide	Treatment of upper respiratory infection, ulcerative colitis	
Eclipta alba	Compositeae	Vringraj	Leave	PA not present others Ecliptine	Inflammation, hernia, bronchitis, asthama, anemia, skin and heart diseases	
Rouwolfia serpentine	Apocynaceae	Sarpagandha	Leave	PA not present others Reserpine, sepentinine	Antihypertensive, hypnotic, reduce blood pressure	[18]
Withania somnifera	Solanaceae	Ashwagandha	Leave	PA not present others Withanolides	Aphrodisiac,liver tonic, treatment of inflammation, bronchitis, asthama, ulcers, insomnia	[19]

II. Material And Methods

2.1 Materials required

Ascorbic acid, sodium nitroprosside, 4- dimethylaminobenzaldehyde, acetic acid, perchloric acid, senecionine, Aluminium silica gel plate. All were obtained from SRl (India).

2.2 Collection of samples

The reported PAs containing plants parts as leaves were collected from local botanical garden and others resources.

2.3 Alkaloids extraction

The plant parts as leaves were isolated from the whole plants. The samples were kept for few days for drying at low temperature. Plant parts were grinded with the help of mixer grinder to obtain powder. Each sample was weighed 1.5 gm with 5% ascorbic acid (40mg) were mixed with small amount sand, separately.

Each sample were shaken for 5 minute and filtered by Whatman's filter paper. Samples are also divided into sample and blank. 12 ml of alkaline 5% sodium nitro prusside reagent was added to the sample tube.

Ehrlich Reagent: Solution was prepared by dissolving 5 gm of 4-methylamino benzaldehyde into 3ml of water, 10ml of 60% perchloric acid and 60ml acetic acid [20].

This Ehrlich reagent was added to the samples containing tube as well as blank containing tube. The tubes were heated on water bath for few minute. The magenta colour was appeared due to unsaturated PAs [20].

2.4 Thin layer chromatography

PAs were estimated using thin layer chromatography [21]. The 20µl alkaloids extracts were applied on aluminium silica gel and developed in mobile phase hexane-ethyl acetate (1:1). After development, chromatogram were dried in air and then sprayed with Ehrlich reagent. After heating at 95°C during 15 minute, pyrrolizidine alkaloids appear as blue or purple spot [22,23]. Senecionine was spotted on TLC for comparing the others spots for analysis by densitometry.

III. Result And Discussion

For the qualitative study and to obtain more accurate results each sample was weighed 1.5, 3, 6 and 9 gm. Due to the increase in the taking amount of sample the extracted PAs will also increased. Therefore the samples colour would not be the same. For the more accurate comparison of colour, the test tubes were given 5, 4, 3, 2, 1 and 0 values based on colour intensity values. The Senecio vulgaris was also kept for a positive control sample. In this experiment the highest colour at lowest amount was given 5 value and the plants which don't have PA was given 0 value. The all samples were compared for colour. The results obtained have been described in Figure 1.0.

For qualitative analysis Thin Layer Chromatography was also used. All plants studies for the presence of Pyrrolizidine Alkaloids showed that some plants contained at least trace amount of PAs but other plants not. The results obtained by TLC screening are in tabulated form Table 2.0.

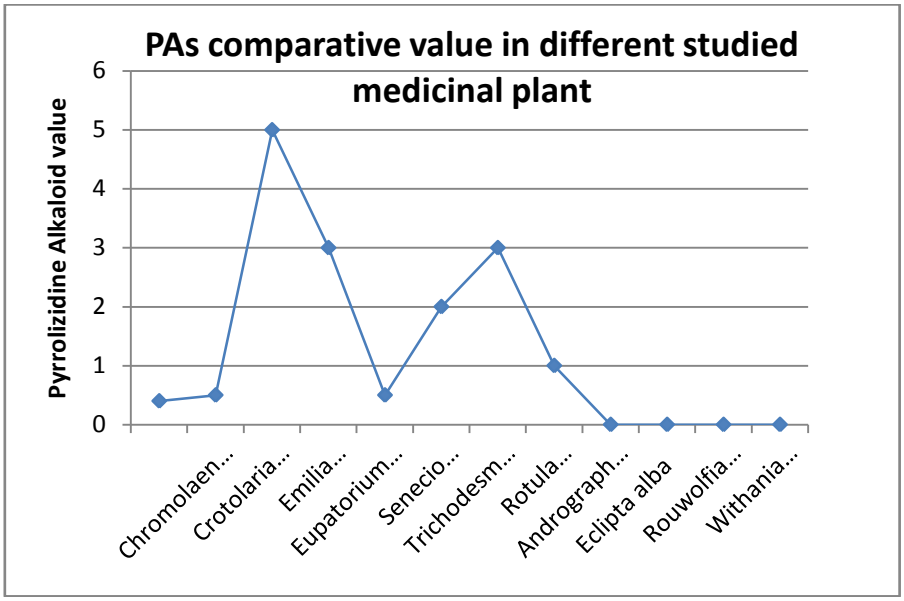

Figure 1.0: PAs comparative value in different studied medicinal plant

Table 2.0: Pyrrolizidine alkaloid-containing plant samples

S. No.	Plant Species	PAs detected	Organ studied	PAs detected	
				colour	Rf
1.	Eupatorium conyzoids	0.03%	Leave	Blue	0.65
2.	Chromolaena odoratam	0.05%	Leave	Blue	0.29
3.	Crotolaria verrucosa	0.29%	Leave	Purple	0.55
4.	Emilia sochifolia	0.2%	Leave	Purple	0.63
5.	Eupatorium triplinerve	0.045%	Leave	Purple	0.65
6.	Senecio vulgaris	0.15%	Leave	Blue	0.48
7.	Trichodesma indicum	0.2%	Leave	Blue	0.33
8.	Rotula aquatic	0.09%	Leave	Purple	0.66
9.	Andrographis paniculata	Not detected			
10.	Eclipta alba	Not detected			
11.	Rouwolfia serpentine	Not detected			
12.	Withania somnifera	Not detected			

Among all studied plant species Crotolaria verrucosa was found to have richest amount of PAs. Emilia sochifolia, Senecio vulgaris and Trichodesma indicum were also found to have more concentrations of PAs. These species also showed 2 or 3 spots on TLC detecting PAs. Eupatorium conyzoids, Chromolaena odoratam, Eupatorium triplinerve and Rotula aquatic were found to have trace amount of PAs. Andrographis paniculata, Eclipta alba, Rouwolfia serpentine and Withania somnifera as known to have no PA also showed no spot or colour in TLC as well as in sample tube.

Plants producing Pyrrolizidine alkaloids are secondary metabolites of plants that are not used by plants but these provide protection and disease prevention. Traditionally used medicinal plants which have PAs are beneficial for health but they have been reported as long term toxicants [4]. These excessive use of these types of medicinal Plants can be dangerous for human health due to hepatotoxicity and neurotoxicity. Some phytopharmaceuticals of several European countries have controlled the use of preparations [24] but these are

still in use by self medication in rural areas or direct marketing by local seller, internet trading. In the present study, some plants showed presence of Pyrrolizidine alkaloids in trace amount.

In the present study the plants were taken from different families as asteraceae, borginaceae, febaceae etc. The plants as Crotolaria verrucosa, Emilia sochifolia, Senecio vulgaris and Trichodesma indicum were found to have more concentrations of PAs. Eupatorium conyzoids has been used as insecticide and nematicide. It contains PAs lycopsamine and echinanin. It can cause liver lesion and tumors. It has been cause of mass poisoning in Ethiopia due to its contamination in grains [8].

Some species of Trichoderma alkaloids have been reported to show remarkable property of primary neurotoxic action. Literature in Russian revealed its intoxication in mice, rabbits and dogs. An outbreak has been also reported in the Samarkand region Uzebekistan in 1950. More than 200 people have been affected due to the consumption of Trichoderma contaminated grains [25]. Chromolaena odorata has been used for medicinal and nutritive purposes in several parts of the world. This species is known to have phytochemical saponin which is associated reduced nutrient utilization in animals. The people who use this plant for medication have high risk of hepatic effect. The study on animal model revealed that consumption of high dose of Chromolaena odorata caused mortality and reduced growth rate [26]. Senecio vulgaris contains senecionin has been listed as a noxious weed. Various reports investigated its hepatotoxicity in cattles and human due to consumption of contaminated grain. Emilia sonchifolia known as Dashapushpam contains tumorigenic Pyrrolizidine alkaloids [27].

Eupatorium is used as folk medicine; it is poisonous to human and livestock. It contains toxic PAs which causes side effects as liver damage, muscular tremor, weakness and constipation. It may be lethal when high doses consumed. Crotolaria species contains PAs crotananine, monocrotaline and cronaburmine and ii is used to promote urination but consumption of regular or high dose caused human poisoning.

IV. Conclusion

It can be concluded that since the plants which contains toxic PAs and are using for preparation of traditional medicine are measured harmful for human and livestock. So the toxicological studies should be done for the permissible and safe level of PAs presence in this herbal medicine. For this Thin Layer Chromatography may be a routine fundamental test for the PAs detection in plant based medicine.

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