SEMI QUANTITATIVE ESTIMATION OF PRUSSIC ACID (HCN) IN SOME MEDICINAL PLANTS IN SOUTHERN REGION OF MAHARASHTRA STATE

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ABSTRACT

Prussic acid or HCN is known to occur in at least 2700 plants in the world in the form of cyanogenic glycosides. It is produced after enzymatic hydrolysis of the cyanogenic glycoside. It is considered to be a defense mechanism of plant against pests. In the present study prussic acid (HCN) content in 35 medicinal plants is analyzed. The semi quantitative estimation of HCN is done with the help of a simple kit. 25 medicinal plants were tested positive for prussic acid.

Keywords: Prussic acid, Cyanide content and Medicinal plants.

INTRODUCTION:

The word 'cyanide' can stir people's emotions. Cyanide C ≡ N exists in various forms in nature as salts of a potassium, sodium and calcium. These are known to be most rapid potent highly poisonous substances in the world, but they are very important in the process of gold recovery from hard rock and extensively used industrially. Cyanide and chemically related compounds are formed, excreted and degraded in nature by hundreds of species of bacteria, algae, fungi and higher plants (Knowles, 1976).

At least 2600 species of higher plants and some microorganisms have been shown to contain one or more of nearly thirty two compounds capable of producing hydrogen cyanide (HCN) or prussic acid. These compounds are mostly derived from amino acids (Seigler, 1976, Moller and Seigler 1999). Among the higher plants at least 2600 are known to be cyanogenic. The process of cyanogenesis is very simple. The cyanogenic glucoside present in the plants under some conditions is converted to sugar and an algycone with the help of β-glycosidase enzyme. In the next step with the help of enzymes the aglycone is converted to HCN and an aldehyde or ketone with the help of another enzyme. Poisoning of live stock by forage Sorghum and other plants is well documented (Mudder, 1997). Occasional accidental poisoning in humans have also been reported (Pentore et.al. 1996). There are many economical important plants highly cynogenic, including white clover, linum, almond, sorghum, the rubber tree and cassava (Tokarnia et.al., 1994; Cheeke, 1995)

In the present study an attempt has been made to detect and estimate amounts of HCN semi quantitatively from some medicinal plants so as to understand the risk factor of consuming these plants, which is highly used against number of ailments in rural areas.

Materials and Methods:

All 35 medicinal plants were collected from different regions of Marathwada particularly Nanded and adjoining District and was immediately identified botanically on the spot in the field by using Flora of Marathwada (Naik, 1998).

After pressing and drying herbarium sheets of these plants are prepared. Field tests for cyanogenic plants were taken wherever possible and even in some cases quantitative estimation was done by the simple picrate paper kit of Bradbury (Bradbury et.al., 1999). All the chemicals and reagents used were purchased from the commercial sources and were of analytical grade.

Semiquantitative estimation of HCN content:

Medicinal plants were tested for the presence of cyanogenic glycosides and release of HCN by simple sodium picrate paper test. The leaf/fruit extract suspected for the presence of cyanogenic compounds were taken in 0.2 M. phosphate buffer pH 10 ml was added. A strip of Whatman filter paper No.1, 5cm x 2 cm. was soaked in sodium picrate solution (25 gms of sodium carbonate and 5 gms of picric acid dissolved in 1000 ml of distilled water) and dried, and it was hanged in glass vials containing the extract of selected medicinal plants to be tested. The colour of the picrate paper was observed after few hours. If the colour changed from yellow to reddish brown, it indicated the presence of HCN in the plant extract and test was positive. Intensity of colour change is related to the amount of the cyanogens present and it is possible to observe colour rating as a measure of concentration. If the test is negative, the tube should be left at room temperature for further 24-48 hours and then re-examined for any non-enzymatic release of HCN.

The quantitative estimation of total cyanide in plant materials is a complicated process. However a simple method and kit for semi-quantitative estimation of HCN is developed by Egan et.al. (1998) and Bradbury et.al. (1999). This kit make use of filter paper caps of enzyme linamarase for release of HCN from glycoside but in the present study instead of it specially prepared caps of filter paper soaked in extract of *Cusuta reflexa* Roxb were used as source of β-glycosidase enzyme. The colour change is compared with the standard colour chart provided by Bradbury, which gives an approximate amount of HCN in ppm.

Table No.1
Detection and estimation of hydrogen cyanide in some medicinal plants by using certain simple techniques.

S. N.	Name of plants	Family	Common name	Phonological stage	Plant part used	Reagent	Amount of HCN in ppm
1	2	3	4	5	6	7	8
1	*Adhatoda zeylanica* Medic.	Acanthecae	ADULSA	Flowering	Leaves	C.C.	10
2	*Aegle marmelos* (L.) Corr.	Rutaceae	BEL	Fruiting	Leaves fruit seed	C.E. C.E. C.E.	20 10 10
3	*Ailanthus excels* Roxb.	Simaroubaceae	MAHARUKH	Vegetative	Leaves	C.C.	10
4	*Argemone mexicana* L.	Parpaveraceae	PIWALA DHOTRA	Flowering	Leaves	C.C.	30
5	*Biophytum sensitivum* (L.) DC.	Oxalidaceae	LAJALU	Flowering	Leaves	C.C.	15
6	*Boerhavia repens* L. var. *diffusa* (L.) Hook. f.	Nyctaginaceae	PUNARNAWA	Flowering	Leaves	C.C.	20
7	*Bombax ceiba* L.	Bombacaceae	KATE SAWAR	Fruiting	Leaves seeds	C.C. C.C.	20 00
8	*Cardiospermum helicacabum* L.	Sapindaceae	KAPAL PHUTI	Fruiting	Leaves Seeds	C.C. C.C.	150 600
9	*Carica papaya* L.	Caricaceae	PAPAI	Fruiting	Leaves Fruits Seeds	HCl HCl HCl	00 00 30
10	*Cayratia trifolia* (L.) Domin	Vitaceae	AMBATVEL	Fruiting	Leaves Fruit	C.C. C.C.	20 20
1	2	3	4	5	6	7	8
11	*Celosia argentea* L.	Amaranthaceae	KARDU	Flowering	Leaves	C.C.	10
12	*Cleome viscosa* L.	Cleomaceae	PIWALI TILWAN	Fruiting	Leaves Fruits	C.C. C.C.	20 20
13	*Curculigo orchioides* Gaertn.	Hypoxidaceae	KALIMUSALI	flowering	Leaves	C.E.	10
14	*Datura metel* L.	Solanaceae	KALA DHOTRA	Fruiting	Leaves Fruit	C.C. C.C.	10 20
15	*Dolichandrone falcata* (Wall. ex DC.) Seem.	Bignoniaceae	MEDH-SHINGI	Vegetative	Leaves	CC.C.	10
16	*Echinops echinatus* Roxb.	Asteraceae	UTAKATARI	Flowering	Leaves	C.E.	10
17	*Enicostema axillare* (Lamak) Raynal	Genetianaceae	NAI	Flowering	Leaves	C.C.	75
18	*Gloriosa superba* L.	Liliaceae	KALLAVI	Flowering	Leaves	C.E.	10
19	*Gymnema sylvestre* (Retz.) R.Br.ex Schult.	Asclepiadaceae	APHUMARI	Flowering	Leaves	C.E.	10
20	*Hemidesmus indicus* (L.) R.Br.	Periplocaceae	ANANT MUL	Vegetative	Leaves	C.C.	10
21	*Hybanthus enneaspermus* (L.) F.Muell.	Violaceae	PURUSH RATAN	Flowering	Leaves	C.C.	50
22	*Lawsonia inermis* L.	lythraceae	MEHANDI	Fruiting	Leaves Fruit	C.C. C.C.	10 10
23	*Leucas cephalotes* (Roth.) Spreng.	Lamiaceae	TUMBA	Flowering	Leaves	HCl	15
24	*Morinda citrifolia* L.	Rubiaceae	BARTONDI	Fruiting	Leaves	C.C.	10

					Fruit	C.C.	00
25	*Mucuna pruriens* (L.) DC.	Fabaceae	KHAJ- KUIRI	Fruiting	Leaves Fruit	C.E. C.E.	20 20
26	*Ocimum tenuiflorum* L	Lamiaceae	TULAS	Flowering	Leaves	C.C.	10
27	*Pergularia daemia* (Forsk.) Choiv	Asclepiadaceae	UTARAN	Fruiting	Leaves Fruit	C.C. C.C.	150 00
28	*Psoralea corylifolia* L.	Fabaceae	BAWACHI	Flowering	Leaves	C.C.	75
29	*Sida acuta* Burm.f.	Malvaceae	BALA, CHIKANA	Flowering	Leaves	C.C.	10
30	*Terminalia bellirica* (Gaertn.) Roxb.	Combrataceae	BEHDA	Fruiting	Leaves Seeds	HCl HCl	20 00
31	*Terminalia catappa* L.	Combretaceae	DASHI BADAM	Fruiting	Leaves Seeds	HCl HCl	00 10
32	*Tinospora cordifolia (*Wild) Miers.	Menispermaceae	GULVEL	Fruiting	Leaves Stem Fruit	C.C. C.C. C.C.	10 00 00
33	*Tribulus terrestris* L.	Zygophyllaceae	SARATA	Fruiting	Leaves Fruit	C.C. C.C.	10 00
34	*Tylophora fasciculate* Ham.	Asclepiadaceae	KHADKI RASNA	Fruiting	Leaves Fruit	HCl HCl	20 10
35	*Vitex negundo* L.	Verbenaceae	NIRGUDI	Flowering	Leaves	HCl	10

C.C. = Cuscuta caps. , C.E. = Cuscuta extract.

RESULTS AND DISCUSSION:

The results of detection and semi-quantitative estimation of HCN (Prussic acid) in medicinal plants are presented in Table No.1. Out of the 35 medicinal plants species tested, 25 were found positive for the presence of cyanogenic glycosides and HCN. The maximum amount of HCN is found to be 600 ppm in seeds of *Cardiospermum helicacabum* L. In fact now it is a well established that the HCN production is a defence mechanism by plants but it is switched on only under particular circumstances.

The permitted dose of cyanide by WHO in foods is 10 ppm, as these plant part are boiled the amount of cyanide is greatly reduced, but even low dose of HCN taken frequently may cause certain side effects (Salkowasi and Penney, 1994).

The negative results are, however to be reconsidered as the HCN production by plants depends upon several internal and external factors as genes and environment (Jones, 1998). Cyanogenesis, however, cannot be considered as a chemotaxonamic parameter, but it is probably an interaction of plants against pests. The study could serve as reference to new studies regarding prussic acid in these plants.

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