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Determination of Cyanide in *Amanitia muscaria* Samples Using Alkaline Picrate Method

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Abstract: Analytical methods were developed for the detection of cyanide in *Amanitia muscaria* samples using the alkaline picrate method. Dried mushroom samples contained in glass bottles were treated with acid solution and maintained at 80°C for 10 min. Hydrogen cyanide vapours librated from the samples reacted with alkaline picrate solution on Whatman filter paper strips to form red-coloured complex on the test strips. The red coloured compound on the test strips were extracted with 50% ethanol solution and the extract absorbance read at 510 nm. A linear relationship was obtained between the ranges of 0-200 µg CN. Cyanide was detected in mushroom samples at the levels of 84-712 µg/10 g sample. Recovery of cyanide from the samples demonstrated that cyanide poisoning could occur in populations due to prolonged intake of toxigenic mushroom samples. Association between cyanide intake from cyanogenic mushroom and the manifestation of severe human diseases were discussed.

Key words: *Amanitia muscaria*, picrate method, cyanide

INTRODUCTION

Mushrooms are a general term applied to the fruiting bodies of saprophytic fungi. Mushrooms have for so many years been used as human food. With the realization of the nutritional value of mushrooms as protein sources, people in several parts of the world now rely on mushroom as an important source of protein. Mushrooms have the potential to convert nutritionally valueless substances into high protein food (Lintzel, 1941; Chang and Hayes, 1978).

Many food sources are highly cyanogenic (Lasch and El-Shawa, 1981; Hall and Rumack, 1986; Akiatonwa *et al*., 1994; Suchard *et al*., 1998). Cyanogenic food sources have caused numerous cases of acute cyanide poisoning. Cyanide is a potent toxin which exerts its toxicity by inhibiting cytochrome oxidase causing a cytotoxic hypoxia. It is toxic to a number of enzyme systems. The main target enzyme is cytochrome C oxidase, the terminal oxidase of the respiratory chain and involves the interaction with ferric ion of cytochrome a_3 (Cummings, 2006).

Amanitia muscaria is a mushroom which occurs singly or in groups and it is found in the forest growing on decaying tress (Alexopoulos, 1962). It is variously classified as edible, as hallucinogenic, as poisonous and as deadly (Tsunoda *et al*., 1989). Mushroom poisoning is caused by the consumption of poisonous mushrooms. Consumption of wild mushroom is wide spread in Africa and the choice of the species to be consumed depends on the tradition. Food poisoning by wild mushrooms including *Amanitia muscaria* occurs very often (Benjamin, 1995; Diaz, 2005). Toxic mushrooms cannot be made nontoxic by cooking, freezing, drying or any other means of processing (Nieminen *et al*., 2006). The only way to avoid food poisoning by toxic mushrooms is to avoid consumption of toxic species. The aim of this work was to develop a rapid and simple spectrophotometric method to screen *Amanitia muscaria* samples collected from different locations in Nsukka for their cyanide levels.

MATERIALS AND METHODS

Sample collection: *Amanitia muscaria* samples were collected from different locations in Nsukka. The mushroom samples were collected into polythene bags and were identified based on the taxonomic descriptions given by Alexopoulos (1962). The samples were dried in an oven at 60°C and ground using a sterile Corona grinder (Medellin, Colombia). The samples were used immediately for cyanide determination.

Determination of cyanide: Alkaline picrate reagent was prepared by a modification of the method described by Williams and Edwards (1980) as follows: Test tubes with 2 ml of 2% KOH and 1 ml of picric acid: Na_2CO_3:H_2O (1:5:200 v/w/v) were prepared. Standard absorbance curves were made with 3 Whatman No 1 papers each with a dimension of 8 x 1 cm. The papers were dipped into the alkaline picrate solution for 15 min. The picrate impregnated papers were removed from the solution and used immediately for cyanide determination. Cyanide solutions containing (50-200 µgKCN/mL) were each prepared in glass bottles. The cyanide was acidified with 20% HCl solution and immediately sealed with 3 picrate impregnated papers. The system was maintained at 80°C in a thermostatic

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water bath (Kotterman, Germany) for 10 min. The bottles were removed from the incubator and kept on the laboratory bench at room temperature (28± 2°C) for 24 h. The red-coloured complex formed on the test strips was eluted with 50% ethanol solution for 30 min and the eluate absorbance was measured at 510 nm using a Spectrumlab 23A spectrophotometer.

Preparation of Samples and cyanide analysis: Whatman number 1 filter papers (8 x 1 cm) were dipped into the alkaline picrate solution and drained free of excess liquid just before use. The filter paper strips were prepared under identical conditions. The samples (10 g per sample) were loaded into glass bottles and acidified with 20% HCl solution. The bottles were sealed with 3 picrate impregnated strips suspended above the acidified samples as the bottles were sealed. The system was maintained at 80°C in a thermostatic water bath (Kotterman, Germany) for 10 min. The bottles were removed from the incubator and left at room temperature (28±2°C) for 24 h. The red-coloured picrate paper strips were removed from the bottles and rinsed in 50% ethanol solution for 30 min and the absorbance of the solution measured at 510 nm using a Spectrumlab 23A spectrophotometer. Cyanide levels of the samples were extrapolated from the standard curve.

RESULTS AND DISCUSSION

Samples of edible mushroom, *Amanita muscaria* were analyzed for their cyanide content using the alkaline picrate method. Data in Table 1 show the cyanide levels of the mushroom samples. The cyanide levels of the samples ranged from 84-712 µg/10 g sample. The formation of red coloured complex by the reactiion of cyanide and alkaline picrate has become a standard agronomic technique used for quantitative estimation of cyanide (Williams and Edwards, 1980). Some procedures used for quantitative estimation of mushroom toxins are elaborate and time consuming and the patient might have recovered by the time the analysis is completed. Therefore development of a simple method for cyanide determination is important for routine analysis of mushroom samples.

A linear relationship was obtained between cyanide concentrations of 0-200 µg HCN equivalents/mL. The values obtained were reproducible and cyanide as low as 1 µg could be detected. The cyanide in the test samples evaporated as HCN with the addition of 20% HCl solution as the sample was maintained at 80°C. Tompsett (1959) reported the volatitization of HCN from cyanogenic biological substances with the addition of mineral acid followed by heating of the sample. The alkaline picrate papers acted as a trapping agent of the liberated HCN (Williams and Edwards, 1980). The HCN liberated slowly changed the colour of the picrate paper strips from orange to red at 28±2°C. The colour was fully developed after 20 h.

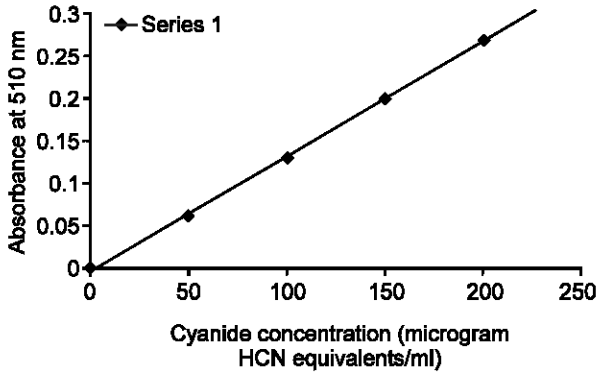

Fig. 1: Calibration curve for cyanide determination by the alkaline picrate method

Table 1: Cyanide contents of *Amanitia muscaria* samples

Sample number	Place of collection	Cyanide content (µg/10 g)
1	Opi	285
2	Ibagwa	522
3	Uzouwani	97
4	Obollo Afor	216
5	Edem	115
6	Aku	586
7	Ukehe	628
8	Ekwegbe	185
9	Ichi	712
10	Orba	669
11	Ikem	84
12	Enugu-Ezike	480
13	Imilike	177

The widespread occurrence of cyanide in many edible mushrooms has been reported (Akiyama *et al*., 2006). The role of cyanogenic foods as etiology of human diseases such as goiter, cretinism, konzo and tropical ataxic neuropathy has been demonstrated from both clinical and epidemiological studies (Tylleskar *et al*., 1991; Banea-Mayambu *et al*., 2000). All humans are susceptible to cyanogenic mushroom poisoning. The poisonous species are ubiguitous. Species of poisonous mushrooms are characterized by their variations in toxin content based on their genetic and growing conditions. Severity of intoxication depends on the amount and type of toxin consumed. From our study, if an individual consumes 1 kg of the mushroom samples, the maximum dose of cyanide intake will be 71.2 mg. The lethal dose of cyanide intoxication of humans is 200-300 mg for an adult human (Akiyama *et al*., 2006). Although the cyanide levels of our samples are low when compared with the lethal dose for humans, epidemiological studies have shown that small doses of cyanide given over a long period of time produced histological changes in the central nervous system (Smith, 1964). The toxicity of cyanide given over

a period of time caused cerebral damage primarily to the basal ganglia (Rachinger *et al*., 2002). Other effects of cyanide poisoning include transcient hyperpnea, headache, dyspnea, central nervous system excitement and progressive histotoxic tissue hypoxia (Hall and Rumack, 1986); konzo, an upper motor neuron disease (Howlett *et al*., 1990; Banea-Mayambu *et al*., 1997; Ernesto *et al*., 2002); cyanide induced parkinsonism (Uitti *et al*., 1985) and chronic neuropathy (Osuntokun 1972; Osuntokun, 1994). Therefore to minimize these health disorders due to cyanide intoxication, development of a simple and rapid analytical procedure to assay cyanogenic mushroom samples becomes necessary especially in developing countries where mushroom has become an important food source.

REFERENCES

Akiatonwa, A., O. Tunwashe and A. Onifade, 1994. Fatal and non-fatal acute poisoning attributed to cassava-based meal. Acta Hort., 375: 285-288.

Akiyama, H., T. Toida, S. Sakai, Y. Amakura, K. Kondo, Y. Sugita-Konishi and T. Maitani, 2006. Determination of cyanide and thiocyanate in Sugihirata ke mushroom using HPLC method with fluorometric detection. J. Health Sci., 52: 73-77.

Alexopoulos, C.J., 1962. Introductory Mycology. 2nd Edn., John Wiley and Sons Inc, New York.

Banea-Mayambu, J.P., T. Tylleskar, K. Tylleskar, M. Gebre-Medlin and H. Rosling, 1997. Geograthical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease, Konzo in former Zaire. Trop. Med. and Int. Health, 2: 1143-1151.

Banea-Mayambu, J.P., T. Tylleskar, K. Tylleskar, M. Gebre-Medlin and H. Rosling, 2000. Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). Ann. Trop. Paediatrics, 20: 34-40.

Benjamin, D.R., 1995. Mushroom: Poisons and Panaceas. WH Freeman, New York.

Chang, S.T. and W.A. Hayes, 1978. The Biology and Cultivation of Edible Mushroom. Academic Press, London.

Cummings, T.F., 2006. The treatment of cyanide poisoning. Occup. Med., 54: 82-85.

Diaz, J.H., 2005. Syndromic diagnosis and management of confirmed mushroom poisonings. Crit. Care Med., 33: 427-436.

Ernesto, M., A.P. Cardoso, D. Nicala, E. Mirione, F. Massaza, J. Cliff, M.R. Haque and J.H. Bradburg, 2002. Persistent konzo and cyanide toxicity from cassava in Northern Mozambigue Acta Tropica, 82: 357-362.

Hall, A. and B.H. Rumack, 1986. Clinical toxicology of cyanide. Ann. Emerg. Med., 15: 1067-1074.

Howlett, W.P., G.R. Brubaker, N. Mlingi and H. Rosling, 1990. Konzo, an epidemic upper motor neuron disease in Tanzania. Brain, 113: 223-235.

Lasch, E.E. and R. El-Shawa, 1981. Multiple cases of cyanide poisoning by apricot kernels in children from Gaza. Pediatrics, 68: 5-7.

Lintzel, W., 1941. The nutritional value of edible mushroom protein, Biochem Acta, 308: 413-419.

Nieminen, P., M. Kirsi and A. Mustoner, 2006. Suspected mycotoxicity of edible wild mushrooms. Exp. Biol. and Med., 231: 221-228.

Osuntokun, B.O., 1972. Chronic cyanide neurotoxicity and neuropathy in Nigerians, Plant Foods for Human Nutr., 2: 215-266.

Osuntokun, B.O., 1994. Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. Acta Hort., 75: 311-321.

Rachinger, J., F.A. Fellner, K. Stieglbauer and J. Trenkler, 2002. MR changes after acute cyanide intoxication. Am. J. Neuroradiol., 23: 1398-1401.

Smith, A.D.M., 1964. Cyanide encephalopathy in man. Lancet, 26: 668-670.

Suchard, J.R., K.L. Wallace and R.D. Gerkin, 1998. Acute cyanide toxicity caused by apricot kernel ingestion: Ann. Emerg. Med., 32: 32:742-744.

Tompsett, S.L., 1959. A note on the determination and identification of cyanide in biological materials. Clin. Chem., 5: 587-591.

Tsunoda, K., N. Inoue, Y. Aoyagi and T. Sugahara, 1989. Simultenous analysis of ibotenic acid and muscioml in toxic mushroom, *Amanitia muscaria* and analytical survey of edible mushroom. J. Food Hyg. Soc. Japan, 34: 12-17.

Tylleskar, T., N. Banea, L. Fresco, L.A. Persson and H. Rosling, 1991. Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. Bull. World Health Organization, 69: 581-589.

Uitti, R.J., A.H. Rajput, E.M. Ashenhurst and B. Rozdilsky, 1985. Cyanide-induced parkinsonism: A clinicopathologic report. Neurol., 35: 921-925.

Williams, H.J. and T.G. Edwards, 1980. Estimation of cyanide with alkaline picrate. J. Sci. Food Agric., 31: 15-22.