EVALUATION OF NICOTINE CONSTITUENT IN NICOTIANA RESTICA

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Abstract - Nicotine is a pyridine alkaloid found in the nightshade family of Solanaceae. Nicotiana tabacum, or cultivated tobacco, is the most commonly grown of all plants in the genus Nicotiana, but it is believed to be a hybrid of other Nicotiana spp . Nicotiana restica is a rainforest plant in the Solanaceae family. The high concentration of Nicotine in its leaves makes it useful for producing pesticides. This study focuses on the detection of Nicotine constituent in plant parts of Nicotiana restica that grows Yerba in Sudan. Plant parts (stem. leaves. fruit and roots) were chromatographically analyzed using TLC and GCMS techniques. Results were compared to same analysis of authentic standards of Nicotine. Nicotine concentration was in range of 0.01 mg/g in stem, fruits and roots; while the concentration in leaves was in range of 0.04 mg/g. The study concluded that Nictiana rusica specimens were very ritch of Nicotine which can make it a good material for medical and chemical industries.

Keywords - Solanaceae, Alkaloids, Nicotine, Nicotine Production, tobacco, GCMS, TLC, medical and chemical industries.

I. INTRODUCTION

Nicotine is pyridine alkaloid found in the nightshade family of (solanaceae), and natural in all parts of the tobacco plant, with a higher concentration in leaves, which constitutes 0.3 to 5% of plant dry weight .with biosynthesis taking place in the roots and accumulating in the leaves. Nicotine found in tobacco, tomatoes, potatoes, green papers, eggplants and other, it is also found in coca leaves. Plants, especially tobacco, used nicotine to defend themselves against insect (ujvary istvan 1999). Nicotine has a lot of therapeutic uses. There's growing evidence that it may be useful in treating Parkinson's disease, Alzheimer's, Nicotine was widely used as an insecticide (Rodgman 2009).

Nicotine the molecular formula C10H14N12 and molar mass of 162 g/mol (Figure1) .It is colorless to pale yellow, very hygroscopic, oily liquid with an unpleasant pungent odor. It gradually becomes brown on exposure to air or light. Nicotine is very soluble in ethanol, chloroform, petroleum ether, kerosene, oils and ether. (Anthony et al 2011).

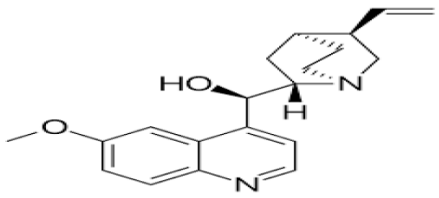


Figure 1 Nicotine chemical structure

For Nicotine production Tobacco dust/ Tobacco Rava and lime are mixed together (using 10% lime). Water is added into the barrel from the top and is allowed to percolate through the dust. The water dissolves all the Nicotine present in the dust and is collected into a sump (Broth). This Nicotinised water (Broth) is then subjected to liquid - liquid extraction using kerosene as solvent. (NPCS Board of Food Technologists 2019)

Nicotine poses several health hazards. There is an increased risk of cardiovascular, respiratory, gastrointestinal disorders. There is decreased immune response and it also poses ill impacts on the reproductive health. It affects the cell proliferation, oxidative stress, apoptosis, and DNA mutation by various mechanisms which lead to cancer. It also affects the tumor proliferation and metastasis and causes resistance to chemo and radio therapeutic agents. The use of nicotine needs regulation. The sale of nicotine should be under supervision of trained medical personnel (Mishra et al. 2015). Philippa 2017 reported that nicotine is readily absorbed into breast milk from the mother's blood, However another study found ten times as much cotinine (a metabolite of nicotine with a much longer half life that can be measured in blood, urine, saliva, hair and nails) in breastfed babies of smoking mothers (Bajanowski et al. 2007).

The Solanaceae consists of about 98 genera and some 2,700 species, with a great diversity of habitats, morphology and ecology. The Ncotiana genera includs about 76 spechies (Olmstead 2007).

Nicotiana tabacum, or cultivated tobacco, is the most commonly grown of all plants in the genus Nicotiana, and its leaves are commercially used in many countries to be processed into tobacco. Research is ongoing into its ancestry among wild Nicotiana species, but it is believed to be a hybrid of Nicotiana sylvestris, Nicotiana tomentosiformis, and possibly Nicotiana otophora. (Ren et al 2001)

Nicotiana restica is a rainforest plant in the Solanaceae family. It is a very potent variety of tobacco. The high concentration of Nicotine in its leaves makes it useful for producing pesticides. It is often used for entheogenic purposes by South American shamans. It contains up to nine times more nicotine than common species of Nicotiana such as Nicotiana tabacum (common tobacco). It is smoked in cigars or used as an enema (Stanfill et al 2015). It is

a hardy plant (figure 2) and can be grown in most of Russia (as opposed to N. virginiana which requires a warm climate), it was more readily and cheaply available, and did not depend on transport in a country with an underdeveloped road network and climatic portage problems. N. rustica leaves have a Nicotine content as high as 9%, whereas N. tabacum leaves contain about 1 to 3%.(Ley, Willy 1965).



Figure 2 Nicotiana restica

This study focuses on the detection of nicotine content in Nicotiana restica which grows wild in Sudan and compares it to the main source of nicotine Nicotiana tabacum.

II. MATERIAL AND METHOD

Materials:

- Nicotiana restica samples collected from Halfa, Northern state, Sudan.
- Standard sample of Nicotine <99% liquid purchased from sigma-Aldrich.

Samples preparation:

Plant Part (roots, stems, leaves and fruits) were collected, dried at room temperature and grained into powder form.

Nicotine standard were prepared by adding 1 ml of methanol to 1 ml of Nicotine standard.

Extraction:

1g of herbal powder of each sample of plant part of targeted plant were weighed; 1ml of chloroform was added to each sample and left for 24 hour at room temperature. The suspensions were filtered (Anthony et al 2005).

Thin layer analysis (TLC):

Samples of plant extract were loaded in TLC plates. A control sample of Nicotine was loaded in the plate beside the plant samples,. The plates of TLC analysis were but in TC system (mobile phase chloroform 90% methanol %10 in saturated condition) the mobile phase run for 10 cm. The RF was observed under ultraviolet radiation at a wavelength of 254nm (Anthony et al 2005).

Gas chromatography analysis with mass spectrometer GC MS:

Samples were evaporated under Nitrogen, the dried residues dissolved in solution of (methanol: acetic acid 9:1) then dried using anhydrous sodium sulphate.

The samples were injected in GCMS chromatograph (Shimatzu 4080). The Injection volume was 1 ul, the start temperature of the column was 80c hold for 1 minute, then increase to 200 c at a rate of 15dgree per minute and hold for 1 minute.

Then increased to 260c at a rate of 10 degree per minute and hold for 1 minute, and then increased to290c at a rate of 10 degree for 2 minute, the result were showed as chromatogram and Mass spectrum and were compared to Nist library attached with device.

III. RESULTS AND DISCUSSION

Results of TLC analysis:

At 10 cm run for the mobile phase Rate of flow RF of Nicotine standard was 3.6. All plant parts samples of Nicotiana restica were showed positive result for Nicotine.

Result of GCMS analysis:

GCMS analysis of Nicotine standards:

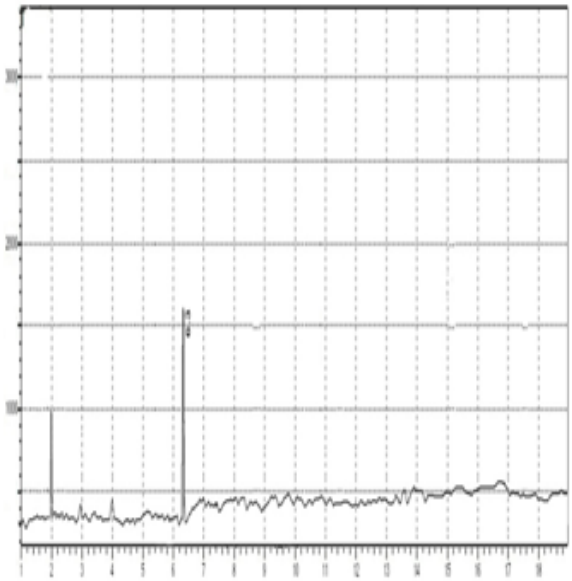


Figure 3 GC chromatogram of Nicotine standard

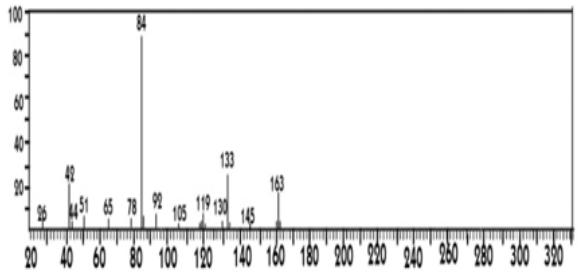


Figure 4 Mss Spectra of Nicotine standard

The peak height of Nicotine standard chromatogram was 1652376.

For Nicotine standard the Retention Time of the peak was 6.3 (Figure 3). The mass spectrum showed highest mass fragment value of 163 in a similarity with value of the molecular weight of Nicotine (Figure 4).

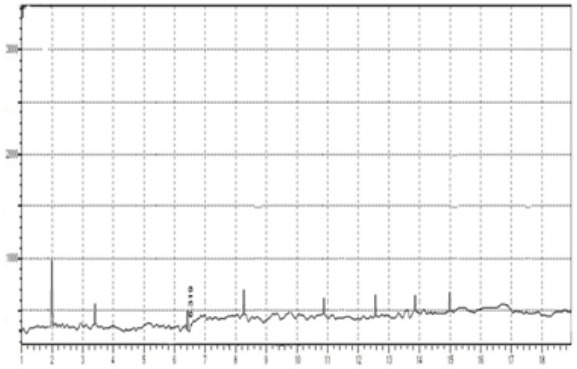


Figure 5 chromatogram of Nicotina restica stem analysis

Results of analysis of Nicotina restica plant parts:

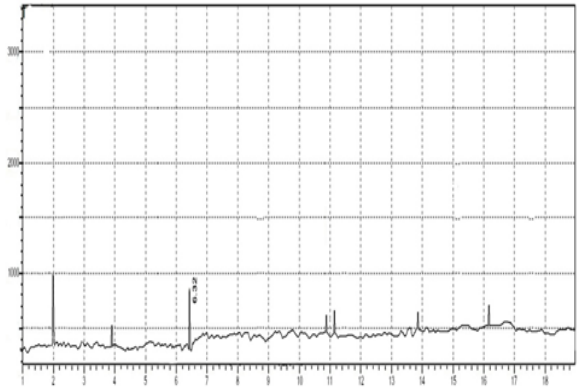


Figure 6 chromatogram of Nicotina restica leaves analysis

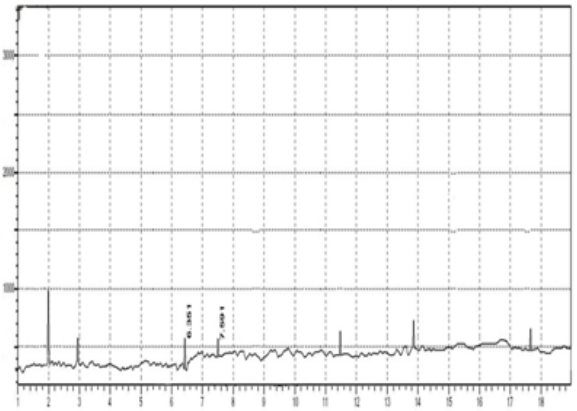


Figure 7 chromatogram of Nicotina restica fruit analysis

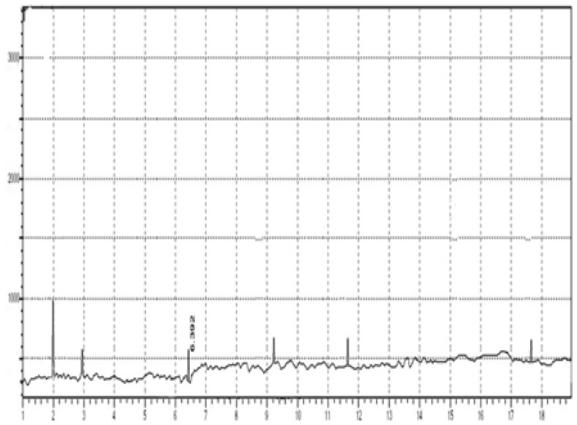


Figure 8 chromatogram of Nicotina restica root analysis

Plant part	Nicotine Peak height
Stem	29887
Leaves	74277
Fruits	30322
Roots	29243

Table 1 peak heights of Nicotine in N. restica plant part samples

Figures 5, 6, 7 and 8 show chromatograms of analysis of stem, leave, fruit and root samples of N. restica, all chromatograms show peaks in Retention Time around 6.3 minute, all mass spectra of those peaks are in agreement with the mass spectrum of Nicotine standard, there was no doubt Nicotine is present in all samples.

Plant part	Nicotine Concentration mg/g
Stem	0.01820
Leaves	0.04450
Fruits	0.01816
Roots	0.01752

Table 2 Concentrations of Nicotine in N. resteca plant parts

After calculation, Nicotine concentrations in Nicotiana restica was showen in table (2), it is manly Nicotine in stem, fruits and roots in range of 0.02 mg/g, the concentration in leaves was in range of 0.04 mg/g , this concentration is higher than Nicotiana tobacum which relative to what was stated by (Katherine 2008).

IV. CONCLUSION

Nictiana rusica specimens were very rich in Nicotine which can make it a good material for medical and chemical industries although it is free of toxic addictive alkaloids.

This study is recommending to direct industrial capital to benefit from these land resources and to carry out more scientific and economic studies.

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