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Phytochemical Screening of Tobacco (*Nicotiana Sylvestris)*

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A B S T R A C T

Nature has bestowed on us a very rich botanical wealth and a large number of distinct types of plants. Medicinal plants which grow in different parts of the world are known to be the richest resource of drugs of traditional systems of medicine, modern medicine, folk medicine and pharmaceuticals. The parts of Nicotiana sylvestris plant have been known to possess a wide range of phytochemical properties. This investigation focused on the phytochemical properties of Nicotiana sylvestris using three different extracts (aqueous extracts, acetic extract, and ethanol extracts). The plant material used in this work was collected from School to land, Rumuodumaya, Rivers State, Port Harcourt. The tannin, alkaloid, flavonoid, phytate, saponin, steroid, phenol and oxalate composition of the leaves of Nicotiana sylvestris were analysed quantitatively and qualitatively using Boham, Kocipal-Abyazan, Harbone and Follin-Dennis methods of analysis respectively. The phytochemical screening carried out showed that the leaves of Nicotiana sylvestris contains different phytochemical constituents in its quantitative analysis: Tannin (2.0mg/kg), Saponin (1.85%), Cyanogenic glycoside (1.45mg/kg), Phenol (93.5mg/kg), Alkaloid (11.4%), Flavonoid (3.82%), Oxalate (2.13%) and also its qualitative analysis: Tannin (+), Saponin (++), Cyanogenic glycoside (+), Phenol (++), Alkaloids (++), Flavonoids (++), Phytate (-), Oxalate (+). It was observed that there are plant-based bioactive compounds produced by plants called phytochemicals and the phytochemical screening of Nicotiana sylvestris leaf extract reveals the presence of Tannin, Saponin, Cyanogenic glycoside, Phenol, Alkaloid, Flavonoid, Oxalate in their different quantities using quantitative and qualitative analysis. Therefore phytochemical screening of Nicotiana sylvestris has helped in the pharmaceutical discovery of bioactive agents that can be used in the synthesis of plant driven medicines.

Keywords: Phytochemical, Screening, *Nicotiana sylvestris*, Tobacco

INTRODUCTION

Phytochemicals are the chemicals derived from leaves, flowers, seeds, barks, roots and pulps of plants. They play a key role against a number of diseases such as asthma, arthritis, cancers and so on. The main components of plant-derived phytochemicals are alkaloids, flavonoids, glycosides, tannins, saponins, phenols, and terpenoids (Saxena et al., 2013). Phytochemistry takes into consideration the natural composition of these metabolites, the biosynthetic pathways functions, medicinal, industrial and commercial application. Nicotine is the predominant alkaloid in Nicotiana sylvestris and unlike for most Nicotiana species in which the roots contain higher quantities of alkaloids compared with the leaves, the total alkaloid content in dry Nicotiana sylvestris leaves is the highest (2.96%) in the genus and only 0.786% in roots

(Heemann et al.,1983).

The nicotine content of Nicotiana sylvestris (82% of 4.8mg/g total alkaloids) was found to be much higher than the nicotine content of Nicotiana tomentosiformis (6% of 0.5mg/g total alkaloids), and this could be the driving force behind the favorable allotetraploidy between Nicotiana sylvestris and other Nicotiana species (Clarkson et al., 2004).

The Nicotiana genus is a rich source of terpenoids, the biosynthesis of which has been reviewed previously (Clarkson et al., 2004). Terpenoids play a significant role as attractants to a number of insects that pollinate Nicotiana sylvestris (Eich, 2008). Another peculiar property of Nicotiana species is their high susceptibility to accumulate cadmium as well as

other heavy metals (Heemann et al., 1983).

There are huge number of herbal medicines described in Ayurvedic and other alternative traditional medicines whose popularity and use in uplifting the general health of common people is still not so efficient because of several reasons. There are so many herbal medicines either individually or in combination

which are being used in various medical treatments for the cure of different ailments. Holistic approach of Ayurveda in regard to preventive, promotive and curative measures with due consideration of health and disease is well established. (Eich, 2008). Tobacco is an agricultural product processed from the leaves of plants in the genus

Nicotiana. It can be consumed, used as a pesticide and, in the form of nicotine titrate, used in some medicines.

It is most commonly used as a drug, and is a valuable cash crop for countries such as Cuba, India, China,

and the United States. (Yemets et al.,2008). Tobacco is a name for any plant of the genus Nicotiana ofthe Solanaceae family (night shade family) and for the product manufactured from the leaf and used in cigars and cigarettes, snuff, and pipe and chewing tobacco, this plants are also used in plant bioengineering, and some of the more than 70 species are grown as ornamentals (Rawat and Mali, 2013).

MATERIALS AND METHODS

2.1.2 Chemicals and reagents used

- Crucible
- Pipette
- Distillation pipe
- Filter paper
- Beaker
- Conical flask
- Labelling tape
- Lab coat
- Capillary tube
- Foil
- Mortar and pestle
- Test tube
- Centrifuges

2.2 Methods

2.2.1 Plant materials

Nicotiana sylvestris was collected from Rumuodumaya, School to Land, Obio/Akpor Local Government, Rivers State, Nigeria.

Plate 2.1: Freshly cut leaf of *Nicotiana sylvestris*

2.2.2 Extraction and Analysis Procedure

The leaves of *Nicotiana sylvestris* were manually separated from the stem and the sample were crushed with a crucible into a paste like form. The crushed sample were measured for each of the analysis carried out.

Plate 2.2: Crushed sampled of *Nicotiana sylvestris*

QUANTITATIVE ANALYSIS OF PLANT PARTCONSTITUENT OF *Nicotiana sylvestris*

Test for tannin

One gram (1g) of the crushed sample of *Nicotiana sylvestris* were weighed into a 100ml conical flask. And 100ml of water were added, shaken and filtered whilst warm, through a filter paper in a 50ml volumetric flask. A paper blank test were done and 0.3ml tannin acid were collected using a pipette into a 50ml volumetric flask to give a standard range from 0 – 0.3mg tannin acid. A suitable aliquot of the sample were pipetted into a 50ml Volumetric flask. Water were added until the flask is two-third (2/3) full and a 2.5ml follin-dennis reagent were added and 10ml sodium carbonate solution. The volume of the mixture were diluted and mixed, it was allowed to stand in a water bath of 25°C for 20 minutes. The optical density were measured at 760nm and blank test was carried out.

Test for alkaloid (Determination using Harbone (1973))

The crushed plant part sample of 2.5g were weighed into 250ml of 20% acetic acid and 20ml ethanol were added, covered and allowed to stand for 4hours, this was filtered out and the extract were concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide were added dropwise to the extract until the precipitation were completed. The whole was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which were dried and weighed.

Test for flavonoid (Determination using Boham and Kocipaibyazan (1970))

The crushed plant part sample of 5g were extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. 20ml of water were added to make it 100ml then shaken. The whole solution were filtered through whatman filter paper No. 22 (125mm) and the filtrate were later transferred into a crucible and allowed to evaporate into dryness over the water bath and weighed to a constant weight.

Test for cynogenic glycoside

14g of crushed plant sample were weighed into a distillation flask. 20ml distilled water were added and the sample were allowed to stand overnight for proper hydrolysis to be attended. The sample were distilled into 20ml sodium hydroxide containing 0.5g crystal. The distillate were titrated with 0.02ml 5% potassium iodine and 1ml 6%N ammonia hydroxide solution to a permanent turbidity.

Test for phytate

1g of the crushed plant sample were weighed and 100ml of water were added, shaken and put to boil. 50ml distilled water were made and 15ml were taken from the distilled water, phenopthaline were added to it and neutralized with 0.5m Na_2OH which turns purple and make slightly acidic with 0.17m Hcl_2 (turns colourless). 50ml distilled water were made and 10ml aliquot and 4ml $FeCl_3$ were added into a centrifuge table and heated for 15mins at 100°C. it was allowed to cool, centrifuge and the liquid were decanted. 2ml water and heat were added to the residue and allowed to boil for few minutes at 100°C, 2ml 0.5m NaOH were added and allowed to heat for 15minutes and then filtered into a conical flask. The filter paper was washed with hot water and the flask was retained.

Test for phenol

1g of the crushed plant sample were weighed and 100ml of distilled water were added and allowed to boil for 30 minutes. 2.5ml aliquot and 5ml of 0.1m NaOH were added into a conical flask and warmed at 50°C and allowed to cool, 2.5ml of 0.05I_2 (Iodine) were added and corked with a foil and allowed to stand at room temperature °C. 0.5ml concentrated HCl_2 were added and titrated to a pale-yellow color with 0.1m $Na_2S_2O_3$, using starch as indicator (3 drops) and titrated again with 0.1m $Na_2S_2O_3$ and blank (Bk) were titrated the same way.

Test for oxalate

10g of crushed plant sample were weighed and 100ml of water were added into the crushed plant sample and were heated with a heating mantle for one hour and allowed to boil**.** 100ml of water were added and later filtered. 25ml of sample were measured into a conical flask and 20ml of 2M H_2SO_4 were added. The sample were heated on a heating mantle and a thermometer was put into it while it was heating till it got to 70°C temperature and then the flask was brought down. The sample were titrated with $KmNO_4$, until it became pink.

Test for flavonoids

4 Drops of concentrated HCl_2 were added to 5ml of the extract after which 0.5g of magnesium were added, development of a pink or magenta – red coloration which indicated the presence of flavonoid were observed. 1ml of 10% NaOH were added to 3ml of the extract. A yellow coloration indicated the presence of flavonoid.

2.2.4 QUALITATIVE ANALYSIS OF PLANT PARTS CONSTITUENT OF Nicotiana sylvestris

Test for flavonoids

About 6ml of 10% dilute ammonia solution were added to a portion of the aqueous literate of the plant extracts, followed by addition of concentrated H_2SO_4. A yellow coloration observed in the extract indicated the presence of flavonoid.

Test for saponins

About 2g of the powdered sample were boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate were mixed with 5ml distilled water and shaken vigorously for stable persistent froth. The frothing were mixed with 3 drops of olive oil, shaken vigorously for uniformity and were then observed for the formation of emulsion.

Test for tannin

About 0.5g of the sample were boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green to blue-black coloration. Presence of brownish green or blue-black coloration confirmed the presence of tannin.

Test for cardiac glycoside

About 5ml of the extracts were treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This were underage with 1ml of concentrated H_2SO_4. A browning of the interface suggests a deoxylsugar characteristics of cardenolodes. A violet ring may appear while in the acetic layer, a greenish ring may form gradually.

Test for terpenoids

About 5ml of each extract were mixed in 2ml chloroform, and concentrated H_2SO_4 (3 ml) were carefully added to form a layer. A reddish-brown coloration of the interface were formed to suggest positive results for the presence of terpenoids.

Test for steroids

About 2ml of acetic anhydride were added to 0.5g ethanol extract of each of the sample with 2ml H_2SO_4. The color changed from violet to blue or green indicating the presence of steroids.

RESULTS AND DISCUSSION

3.1 PHYTOCHEMICAL SCREENING

The phytochemical screening carried out showed different phytochemicals present in the plant and at different composition. It contains phytochemical constituents, including tannins, saponins, cyanogenic glycoside, phenols, alkaloids, flavonoids, phytate, oxalate. In Quantitative analysis, it showed that, the plant has more flavonoids of (3.82%) and alkaloid of (11.4%), in Qualitative it has more saponin (++), phenols (++), alkaloids (++) and flavonoid (++). All these are the phytochemicals present in *Nicotiana sylvestris.*

Table 1: Quantitative composition in *Nicotiana Sylvestris*

Quantitative composition	*Nicotiana Sylvestris*
Tanin	2.0 mg/kg

Saponin	1.85%
Cynogenic glycoside	1.45mg/kg
Phenol	93.5 m g/kg
Alkaloids	11.4%
Flavonoids	3.82%
Phytate	_
Oxalate	2.13%

Table 2: Qualtitative composition in *Nicotiana Sylvestris*

Qualitative compound	*Nicotiana Sylvestris*
Tanin	+
Saponin	++
Cynogenic glycoside	+
Phenol	++
Alkaloids	++
Flavonoids	++
Phytate	_
Oxalate	+

Note**:** **+** is insignificantly present

++ is moderately present

+++ is highly present.

The phytochemical screening of Nicotiana sylvestris indicated that it had alkaloid, tannin, flavonoid, cyanogenic glycoside, phytate, phenol, oxalate, saponins, cynogenic glycoside, terpenoids, steroids (Sallaud et al., 2012). Which are present in different quantities in the leaf of the plant, which were determined using quantitative and qualitative analysis.

The presence of alkaloid showed its use in protecting plants against micro-organisms (antibacterial and antifungal activities), insects and herbivores etc. And also, in pharmacological activities in treatment of hypertension, malaria, helps in preventing cancer and also has stimulant properties such as caffeine, nicotine and morphine which are used as an analgesic and quinine as antimalarial drug (Lasztity et al., 1998). Tannins, alkaloid and flavonoid are phenolic compounds with powerful antioxidant properties that act as free radical, preventing and mitigating the damage they cause (Schofield et al., 2001).

It's terpenoid content is useful in industries and pharmaceuticals because of its use as essential oils, flavors and fragrances in food and cosmetics. All these benefits and more are related to the phytochemical content of Nicotiana sylvestris which makes it a unique and important plant for food, pharmacological and industrial purposes.

Phytochemical Properties of Tobacco leaf contains several pyridine alkaloids, the principal one being a liquid alkaloid, nicotine. Other alkaloids present include nicoteine, nicotimine, anabaine anatallineand nornicotine. It also contains a high percentage of organic acids. Leaves also contain glucosides, tahacinin, tahacilin and isoquercitrin, 1-quinic, chlorogenic, caffeic and oxalic acids. They also contain terpenic and carcinogenic substances

(Shaligram e tal., 2004). Anatabine and (+) nornicotine have been isolated fromroots. Quercetin3,3´- dimethyl ether

and quercetin-3-ether have been isolated from flowers. Three new gibberellins-nicotiana α, β and γ along with gibberllins A and A3 have been isolated from shoot apices and flower buds. Seed contains cycloartanol, cycloartenol 24-daturadiol and solavetivone. Cholesterol, cholest-7-enol, 24-methylenecholesterol, campesterol, stigma sterol,sitosterol,

28-isofucosterol, lanosterol, 31-norlanosterol, lanost-8-enol, obtusifoliol, 31norcycloartenol, cycloeucalenol

granisterol,citrostadienol, β-amyrin, lupeol, cycloartanol and 24-methylenecycloartanol have also been reported in seed oil. (Vaidy and Nighantu, 2009).

Nicotine, isolated from leaves of Nicotiana sylvestris were complexed with zinc and studied for their antibacterial activities against ten different strains of Gram-positive and Gram-negative bacteria Results showed that zinc (II) complex of nicotine is more active against different types of bacterial strains as compared to zinc metal salt used for complexation and nicotine alone (Muhammad et al., 2012). Some other activities reported for Nicotiana sylvestris as; analgesic activity, anesthetic activity, angiogenesis inhibition, anti-bacterial activity, anti-convulsantactivities, anti-estrogenic effect, anti-fungal

activity, anti-glaucomic activity, anti-oxidant activity, anti-stress effect anti-viralactivity, aromatase inhibition, arrhythmogenic effect, carcinogenic activity.

CONCLUSION

The results obtained in the study showed that Nicotiana sylvestris contains a variety of phytochemicals, which reveals its uses for various pharmacological and industrial purposes. The plant leaves can be used in treatment of various disorder in human's which includes pain, cardiovascular diseases, prevention of cancer, microbial and fungal infection. The leaves also act as antispasmodics, diuretics, expectorants, irritants and sedatives.

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