ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics
Available Online at www.jddt.in
CODEN: - JDDTBP (Source: - American Chemical Society)
Volume 8, Issue 8, August: 2020, 05-13

Research Article

# PHYTOCHEMICAL INVESTIGATION AND THE EFFECT ON BIOLOGICAL SYSTEMS OF TOBACCO SAMPLE USED AS STIMULANT

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**Article Info:** Received 04 July 2020; Accepted 25 July. 2020
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**Disclosure statement:** *The authors have no conflicts of interest.*

**Abstract:**
India is the second largest consumer of tobacco globally, and accounts for approximately one-sixth of the worlds' tobacco related deaths. The tobacco problem in India is peculiar, with the consumption of variety of smokeless and smoking forms. Unlike other members of the Solanaceae family, such as tomato and potato, which have uncontroversial nutritional role, tobacco plant carries in its leaf's quantities of alkaloids; Nicotine which acts as a stimulant. This present study investigates the physico-chemical and phytochemical parameters, and the biological activity of the stimulating compound present in the North Indian Tobacco product. Analysis was carried out through chromatographic techniques of TLC, GC-MS, UV and IR spectroscopy. The result of the findings shows that tobacco product contains Acetoin, an additive intoxicating compound which has similar effect as Ethanol intoxication with remarkable antioxidant activity.
Keyword: Tobacco, Stimulant, TLC, GC-MS, Acetoin, Antioxidant activity.

## INTRODUCTION

Tobacco is a leading preventable cause of death, killing nearly six million people worldwide each year. India is the second largest consumer of tobacco globally, and accounts for approximately one-sixth of the world's tobacco-related deaths. [1] In India, tobacco is an important commercial crop fetching more than Rs. 4,400 crores of foreign exchange and generates over Rs 14,000 crores, besides giving direct or indirect livelihood to nearly 34 million people. India occupies 2nd place in tobacco production, after China and 2nd in Exports after Brazil. Indian tobacco has an edge over the leading tobacco producing countries in terms of availability of different styles produced with relatively low production costs. [2]

Tobacco smoke is made up of thousands of chemicals, at least 69 chemicals in tobacco smoke are carcinogenic, and cigarette smoking accounts for at least 30 percent of all cancer deaths. [3] The overall rates of death from cancer are twice as high among smokers as nonsmokers, with heavy smokers having a four times greater risk of death from cancer than nonsmokers. It is said that the most principal constituent found in Tobacco is Nicotine. Although nicotine is addictive, but most of the severe health effects of tobacco use comes from other chemicals. All of these risks apply to use of any smoked product, including hookah tobacco.

Now, as nations have begun to fight back and, in some countries, to turn back the epidemic, tobacco companies continue to develop new products to maintain their profits, often disguising these new products in a cloak of attractiveness and reduced harmfulness. The truth is clear that all tobacco products are dangerous and addictive, and every effort should be made to discourage their use in any form.

The present experiment was undertaken to identify, elucidate and isolate the phytochemical constituent responsible for the intoxicating effect of a North Indian tobacco product commonly known as **Munna Tambaku**.

## MATERIALS AND METHODS

### Collection of Plant Material

Plant material was commercially purchased in one of the Northern states in India. Identification and authentication was done at the Department of Chemistry, Loyola College. Chennai, India.

**Chemicals**: The entire chemicals used in the present study are of analytical grade.

### Preparation of Plant Extract

Dried plant material was seized to separate foreign matters and homogenized to a fine coarse powder using an electric blender and then stored in air tight container for further use. 50ml of water was added to 3g plant powder. The mixture was subjected to cold maceration for 72 hours at 210 rpm, and filtered through a sterilized Whatmann No. 1 filter paper. Filtered extract was concentrated to dryness at $38^0$C under reduced pressure. Thus, the obtained dried extract was kept in a dark bottle and stored in the refrigerator. [4]

### PRELIMINARY QUALITATIVE ANALYSIS:

**1. Test for alkaloids:**

**a. Mayer's Test:**

To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test. Appearance of white creamy precipitate indicates the presence of alkaloids.

**b. Wagner's Test:**

A few drops of Wagner's reagent was added to few ml of plant extract along the sides of test tube. A reddish-brown precipitate confirms the test as positive.

**2. Test for Amino Acids:**

The extract (100mg) was dissolved in 10ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for amino acids.

**a. Ninhydrin Test:**

Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) was added to 2ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

**3. Test for Carbohydrates:**

**a. Molish's Test:**

To 2ml of plant sample extract, two drops of alcoholic solution of alpha naphthol was added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

**b. Benedict's Test:**

To 0.5ml of filtrate, 0.5ml of benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

**c. Fehling's Test:**

The extract was hydrolysed with dil.HCl, neutralized with alkali and heated with fehling's A and B solution. Formation of red precipitate indicates the presence of reducing sugars.

**4. Test for Fixed oils and Fats:**

**a. Spot Test:**

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

**b. Saponification Test:**

A few drops of 0.5N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**5. Test For Glycosides:**

50mg of extract was hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests.

**a. Borntrager's Test:**

To 2ml of filtered hydrolysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicates presence of glycosides.

**b. Legal's Test:**

50mg of extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10%. Presence of glycoside is indicated by pink colour.

**6. Test for Phenolic Compounds and Tannins:**

**a. Ferric Chloride Test:**

The extract (50mg) was dissolved in 5ml of distilled water. To this few drop of 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

**b. Gelatin Test:**

The extract (50mg) was dissolved in 5ml of distilled water and 2ml of 1% solution of gelatin containing was added to it. White precipitate indicates the presence of phenolic compounds.

**c. Lead Acetate Test:**

The extract (50mg) was dissolved in distilled water and to this 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

**7. Test for Flavonoids:**

**a. Alkaline Reagent Test:**

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

**b. Magnesium and Hydrochloric Acid Reduction:**

The extract (50mg) was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) was added. If any pink to crimson colour develops, presence of flavonol glucosides is inferred.

**8. Test for Phytosterols:**

**a. Libermann- Burchard's test:**

The extract (50mg) was dissolved in 2ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour change shows the presence of phytosterols.

**b. Salkowski Test:**

The extract was treated with chloroform and filtered. Filtrate was treated with few drops of con. $H_2SO_4$ shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

**9. Test for Proteins:**

The extract (100mg) was dissolved in 10ml of distilled water and filtered through whatmann No.1 filter paper and the filtrate was subjected to test for proteins.

**a. Millon's Test:**

To 2ml of filtrate few drops of Millon's reagent was added. A white precipitate indicates the presence of proteins.

**b. Biuret Test:**

2ml of filtrate was treated with 1 drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic

layer indicates the presence of proteins.

**10. Test for Saponins:**

The extract (50mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

**11. Test for Gums and Mucilages:**

The extract (100mg) was dissolved in 10ml of distilled water and to this 2ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

**12. Test for Coumarins:**

To 5 ml of aqueous extract, residue was dissolved in 1-2 ml of hot distilled water and volume was divided into two parts. To half a volume, 0.5 ml 10% $NH_4OH$ was added. Two spots on filter paper of solution were examined under UV light. Intense fluorescence indicates the presence of coumarins.

**FLUORESCENCE ANALYSIS:**

0.5g of dried powder was taken into clean and dried test tubes. To each tube 5ml of different organic solvents like dil.HCl , dil.$HNO_3$, aq.NaOH, $CHCl_3$, $H_2SO_4$, distilled water were added separately. Then all the tubes were shaken and they were allowed to stand 20-25min. The solutions obtained were observed under the visible light for their characteristic colour reaction and were compared with a standard colour chart and colours were recorded. [5]

**QUANTITATIVE DETERMINATION:**

The percentage of loss of weight of carbon on drying, acid insoluble ash was obtained by employing standard methods of analysis as described in pharmacopeia of India. [6] Carbon and organic matter present in the drug is converted to ash at

temperature 450°C.It mostly contains carbonates, phosphates, silicates and silica. The procedures were

as follows:

**i. Determination of Moisture Content:**

One gram of powder was weighed and dried at 80°C for 6 hours in hot air oven. After 6 hours, the powder was weighed again and difference in weight was determined. The percentage of moisture was calculated.

**ii. Determination of Total Ash Content:**

2gm of dried plant powder was taken in a previously weighed silica crucible and ignited carefully not exceeding dull red heat, until the ash was free from carbon. The crucible was cooled and weighed. The percentage of ash with reference to the air-dried plant was calculated. [6]

**iii. Determination of Acid-Soluble Ash:**

1gm of ash was weighed and 10ml of conc.Sulphuric acid was added to it. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to constant weight and weighed. The percentage of acid-insoluble ash was calculated. [7]

**iv. Determination of Water-Soluble Ash:**

1gm of ash was weighed and 10ml of distilled water was added to it. The mixture was shaked and filtered through less whatmann filter paper. The ash remained in the paper was kept in silica crucible and burnt again in a muffle furnace for 4 hours. The weight of ash was noted and percentage of water-soluble ash was determined. [7]

**QUALITATIVE DETERMINATION OF ASH FOR MINERAL CONSTITUENTS:**

For detection of various inorganic elements in plant ash viz., Fe, Cl, P, S etc., one gram ash material was dissolved in 25ml of 50% HCl for 12 hours and then filtered through filter paper. [9] The filtrate was treated with suitable reagents to identify the presence of elements qualitatively. The experiments were conducted as by the table below:

**Table 1:** Detection of Elements

| S.NO | EXPERIMENTS | OSERVATION | INFERENCE |
|---|---|---|---|
| 1. | Test solution + 2 drops of dil. $HNO_3$ + 3ml of distilled water + few drops of $AgNO_3$ | White precipitate formation | Presence of Chlorine |
| 2. | Test solution + 2 drops of HCl, warmed + 10 drops of $BaCl_2$ | White precipitate of $BaSO_4$ formation | Presence of Sulphur |
| 3. | Test solution + 2 drops of $HNO_3$, boiled + 4 drops of ammonium molybdate + 4 drops of $NH_4OH$ in water bath at 60°C | Yellow precipitate formation | Presence of Phosphorous |
| 4. | Test solution + 3 drops of potassium thiocyanate | Reddish brown colour formation | Presence of Iron |

**DETERMINATION OF WATER-SOLUBLE EXTRACTIVE VALUE:**

5g of the air-dried drug, coarsely powdered was macerated with 100ml of purified water in a closed flask for 24 hours, kept in a mechanical shaker for first 6 hours and allowed to stand for 18 hours. [10] There after filtered rapidly through whatmann filter paper No.41. Evaporated 25ml of the filtrate to dryness in a preweighed flat-bottomed petridish dried at 105°C and weighed. Calculated the % w/w water-soluble extractive value with reference to the air-dried drug as follows:

$$\text{Water soluble extractive value (\%w/w)} = \frac{\text{Weight of residue X 100}}{\text{Volume of extract evaporated X Weight of sample}}$$

**DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE VALUE**

5g of the air-dried drug, coarsely powdered was macerated with 100ml of alcohol (100% or 60%) in a closed flask for 24 hours, kept in a mechanical shaker for 6 hours and allowed to stand for 18 hours. [6] There after filtered rapidly through whatmann filter paper No. 41. Evaporated 25ml of the filtrate to dryness in a preweighed flat-bottomed petridish, dried at 105°C and weighed. The % w/w alcohol soluble extractive value with reference to the air-dried drug was calculated as follows:

$$\text{Alcohol soluble extractive value (\% w/w)} = \frac{\text{Weight of residue X 100}}{\text{Volume of extract evaporated X Weight of sample}}$$

**THIN LAYER CHROMATOGRAPHY:**

TLC is a chromatographic technique which is used for the separation of mixture of compounds. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). Aqueous Sample prepared was spotted on the TLC plate as a single spot with capillary tubes. [11] TLC plate was first viewed in UV chamber and Rf values was calculated. The used mobile phase system (solvent system) was hexane: ethyl acetate (6:4).

**GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY) ANALYSIS**

The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme. [12]

**UV-VIS SPECTRUM ANALYSIS**

The extract was centrifuged at 3000rpm for 10 min and filtered through Whatmann No.1 filter paper. The sample was diluted to 1:10 with the same solvent. The extract was scanned at wavelength ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The peak values of the UV-VIS were recorded. [13]

**FTIR ANALYSIS**

Dried powder (ethanolic extract) of test plant was used for FTIR analysis. 1 mg of the dried powder was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the pellet was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1. [13]

**ANTIOXIDANT ANALYSIS**

Various concentrations of extract (10 µg, 50 µg 100 µg, and 500 µg) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide.

The mixture was incubated at 50ºC for 20 min. Next, 2.5 mL of 10% trichloroacetic acid (w/v) were added, 5 mL of above solution was mixed with 5 mL of distilled water and 1 mL of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant. [14]

**ESTIMATION OF ALKALOIDS:**

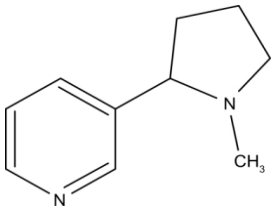
**PROCEDURE:**

0.5, 1.0, 1.5, 2.0 and 2.5 ml (concentration varying from 10 to 50µg) of the standard solution were pipetted out into a series of 25 ml standard flasks and 1.5 ml of the sample was pipetted out into a separate 25 ml standard flask. To all the flasks, including the blank, 1 ml of 0.01 M SPI and 0.5 ml of 0.1 acetic acid was added. Then 10 ml of distilled water was added and kept in the boiling water bath for 10 minutes. Thereafter, 2 ml of 0.01 MBTH into all the flask and boil in a water bath for 2 minutes. All the flasks were cooled and made up to the mark with double distilled water. The blue colour formed was spectrophotometrically measured at λ 630nm. Then the graph was drawn by plotting the concentration of theophylline along the X-axis and the optical density reading along Y-axis. From the standard curve on the graph, unknown sample concentration was calculated. [15]

**ISOLATION OF NICOTINE FROM TOBACCO:**

In a 200 ml beaker, 6g of sample was stirred with 75 ml of 25% NaOH solution for 15 minutes and filtered. Transfer the dark brown filtrate to a 250 ml separating funnel and add 40 ml ether to it. Shake the flask thoroughly and leave it for 5 minutes and allow the layers to separate. Remove the ethereal layer. Repeat

the extraction process again using 40 ml of ether. [16] Combine the two organic layers and distill off the ether on steam bath. Transfer the residual oily portion in a porcelain dish and evaporate off the residual ether. The thick oily residue in the porcelain dish is nicotine, b.p. 247°C.




**Nicotine**

**RESULTS AND DISCUSSION:**

The physico-chemical properties revealed the moisture content of extract to be 12.8%. Total Ash value of plant material indicated the amount of minerals and earthly materials attached to the plant material and its value was calculated to be 67.75%. The amount of acid soluble Ash present in the plant was 33% and the water –soluble Ash was observed to be 56.2% (Table 1).

**Table 1:** Physico-chemical properties

| TEST PARAMETERS | RESULTS (w/w) |
|---|---|
| Moisture content | 12.8% |
| Total ash | 67.7% |
| Acid insoluble ash | 33% |
| Water soluble ash | 56.2% |
| **Extractive values** | |
| Water soluble extractive value | 13.6% |
| Alcohol soluble extractive value | 12% |

**Fluorescent Analysis**

The fluorescence analysis of plant material showed dark green under visible light when the leaf powder was treated with HCl and NaOH Similarly, the leaf powder showed green colour under same light conditions in treatment with $CHCl_3$, $HNO_3$, $H_2SO_4$ and $H_2O$. Brown was observed under UV light for all chemical treatments except for when the leaf powder was treated with $HNO_3$. When the leaf powder was observed under UV light at higher wavelength, it showed varied colour viz., green, pale green, dark green, orange and violet under different chemical treatments.

**Table 2:** Fluorescence analysis

| Particulars of Treatment | Colour under visible Light | Colour under UV light | |
|---|---|---|---|
| | | 254nm | 366nm |
| Powder+dil. HCl | Dark green | Brown | Green |
| Powder+$CHCl_3$ | Green | Brown | Orange |
| Powder+$HNO_3$ | Green | Pink | Violet |
| Powder+$H_2SO_4$ | Green | Brown | Dark green |
| Powder+NaOH | Dark green | Brown | Green |
| Powder+$H_2O$ | Green | Brown | Pale green |

Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostic evaluation.

**Mineral Test**

**Table 3:** Detection of Minerals

| S.No | Minerals | Inference |
|---|---|---|
| 1. | Chlorine | + |
| 2. | Sulphur | + |
| 3. | Phosphorous | + |
| 4. | Iron | - |

Mineral test reveals the presence of Chlorine, Sulphur and Phosphorus with absence of Iron.

**Phytochemical Studies**

The aqueous extract of *Nicotiana tabacum* was subjected to preliminary phytochemical screening for the detection of various plant constituents and results given in Table 4.

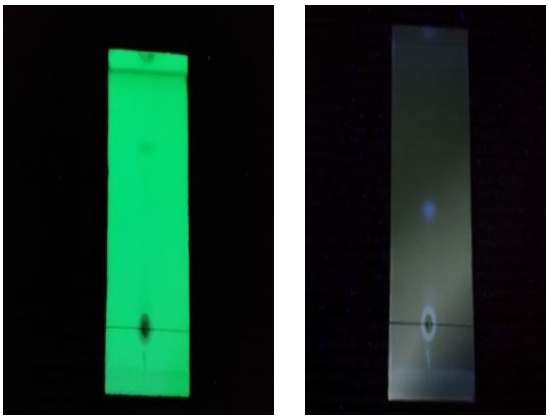
**Table 4:** Phytochemical Screening

| S.No | TEST | AQUEOUS EXTRACT |
|---|---|---|
| 1. | Alkaloids | |
| | a. Mayer's test | + |
| | b. Wagner's test | + |
| 2. | Amino acids | |
| | a. Ninhydrin test | - |
| 3. | Carbohydrates | |
| | a. Molisch's test | - |
| | b. Benedict's test | + |
| | c. Fehling's test | - |
| 4. | Test for Fixed oils and Fats | |
| | a. Spot test | - |
| | b. Saponification | - |
| 5. | Test for Glycosides | |
| | a. Borntrager's test | - |
| | b. Legal's test | - |
| 6. | Phenolic compounds and Tannins | |
| | a. Ferric chloride test | - |
| | b Gelatin test | - |
| | c. Lead acetate test | - |
| 7. | Test for Flavonoids | |
| | a. Alkaline reagent test | - |
| | b. Magnesium and Hydrochloric acid reduction | - |
| 8. | Test for Phytosterols | |
| | a. Libermann-Burchard's test | - |
| | b. Salkowski test | + |
| 9. | Test for Proteins | |
| | a. Millon's test | + |
| | b. Biuret test | - |
| 10. | Test for saponins | - |
| 11. | Test for Gums and Mucilages | - |
| 12. | Test for Coumarins | - |
| **(+)=Presence** | **(-) = Absence** | |

Phytochemical investigation of aqueous extract of *Tambaku* sample revealed the presence of Alkaloids with mild presence of carbohydrates, phytosterol and proteins. Alkaloids have shown to have a major target on the Nervous system since they structurally resemble neuro-transmitters. Because of their pharmacological properties, some alkaloids can be used as medicine to treat health disorders, infections and even cancer.

**THIN-LAYER CHROMATOGRAPHY**

TLC results showed the formation of 1 distinct spot with a solvent combination of hexane and ethyl acetate in the ratio 6:4 at 254nm and 366nm respectively. This result confirms the presence of one bio-active compound in plant extract. The Rf value was found to be 0.7.



**6:4 ratio 254nm** **6:4 ratio 366nm**

**Figure 1:** TLC profile of hexane: ethyl acetate

**ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROSCOPY**

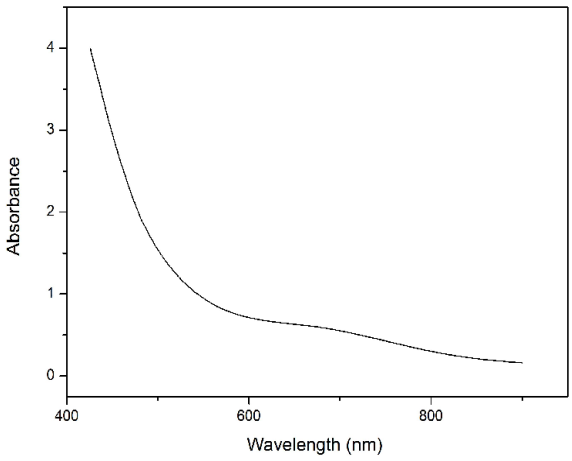



**Figure:** 2 UV-Vis spectroscopy analysis

Λmax was observed at wavelength near 450nm and that indicated the presence of one bio-active compound.

**FOURIER TRANSFORM INFRA RED ANALYSIS**

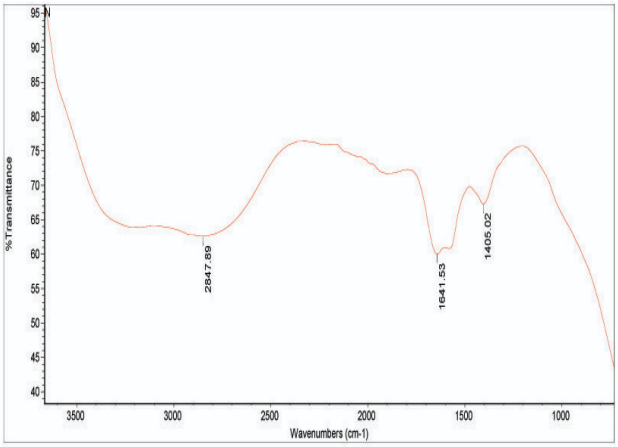



**Figure 3:** Infra-Red Spectroscopy Analysis

The Characteristic absorption bands of the given extract were found. The following table gives the assignment of bands to characteristic functional groups.

**Table 5:** FTIR spectral peak values and functional groups obtained for the leaf extract of *Munna Tambaku*

| Extract prepared | Peak values | Functional groups |
|---|---|---|
| Aqueous | 1405.02 | C-H group (Rocking) |
| | 1641.53 | C=O group (Aromatic) |
| | 2847.89 | CH2 group (asymmetric |
| | | stretching) |

**GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY) ANALYSIS**

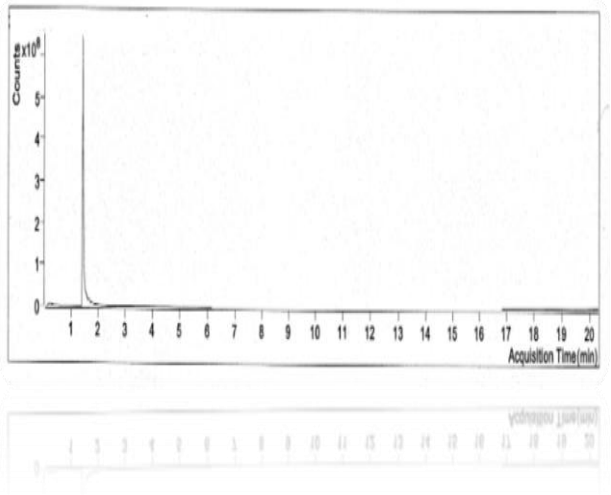



**Fig. 4** GC-MS of Tobacco Product

The graph above shows the presence of one active compound which was identified to be Acetoin.

**ANTIOXIDANT ACTIVITY**

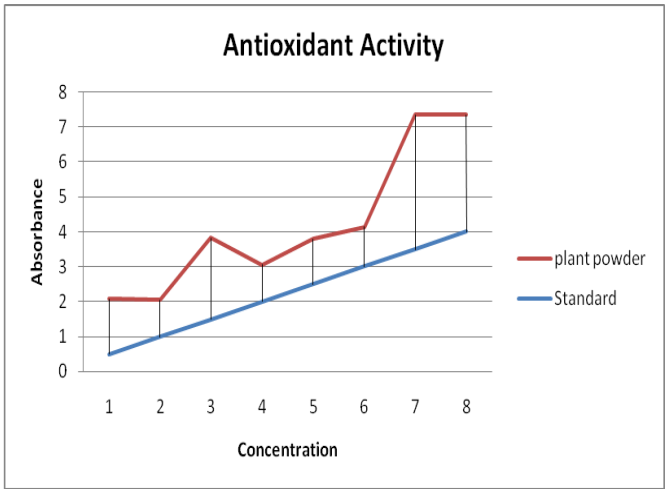



**Figure 5:** Antioxidant activity of Tobacco product

The extract has a remarkable antioxidant activity in fact a higher antioxidant activity of the extract exceeding the antioxidant activity of the standard was recorded in the graph. As the concentration of the extract increased the free radicals were scavenged effectively then the concentration of the extract was extremely high. The steady increasing trend was observed which indicated an optimum level of antioxidant activity of the extract.

**ESTIMATION OF ALKALOID**

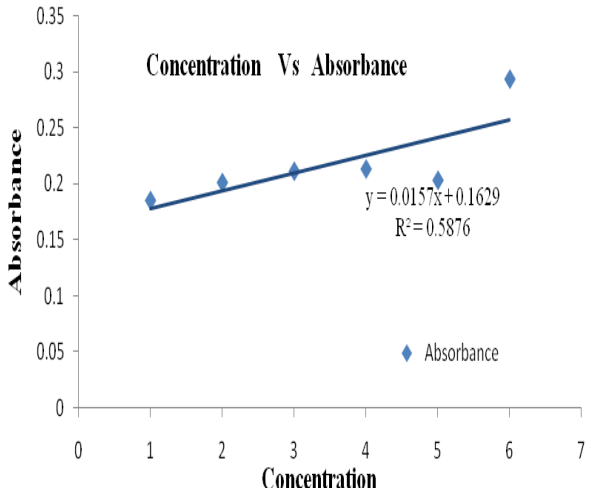



**Figure 6:** Total estimation of Alkaloid present in sample is calculated to be 4.65mg.

**DISCUSSION**

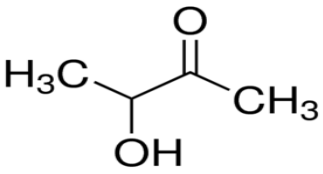
The knowledge and improvement in the quality control and standardization of herbal plant products has led to the development of effective quality medicine from plants. The present investigation hits the pharmacognostic evaluation, phytochemical screening and antioxidant activity for judging acceptability or rejection of commercially available tobacco product. Important parameter for evaluation of crude drug by physical constant evaluation is to detect the adulteration or improper handling of drugs[17]. Evaluation of crude drugs is the ash value, acid insoluble ash value and water-soluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica.[18] The ash value of tobacco product is 67.7%.This ash value is indicative of the impurities present in the drug; this value is also one of the diagnostic parameters of the drug. In the present study, the plant product powder has more water-soluble ash value than acid insoluble ash value.

Fluorescence is the phenomenon to exhibit various colour due to chemical constituents present in the plant material. The fluorescence colour is specific for each phyto-compound. The non-fluorescent compound may be fluorescence if it is mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of pharmaceutical samples.[19] In the present study, the powdered tobacco product emitted medium range of color under daylight and under UV light after treatment with various acids, alkalis & other reagents. Florescence analysis of powders gives a clue if powder is in adulteration thus can be used as a diagnostic tool for testing the adulteration. This procedure is pre-requisite before going for detailed phyto chemical investigation. The phytochemical screening of plant extract was undertaken for the identification of different chemical constituents' present. The aqueous extract of tobacco product obtained by process of maceration has shown the presence of Alkaloids and carbohydrates.

Humans require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water. [20] Inorganic elements play an important role in physiological process involved in human health. Test result reveals the presence of Calcium, sulphur and Phosporus. Every constituent plays an important role and deficiency of any one of mineral constituent may lead to abnormal developments in the body.

Thin layer chromatography of extract also showed one spot at UV wavelength of 256nm and 366nm owing the presence of one active compound present in plant extract. This confirms tobacco product to have a proven Antispasmodic, diuretic and expectorant activity. [21] Isolation of Compound was carried out and subjected to further analysis. The UV results according to figure 2 confirms the presence of one compound at wavelength of 450nm. FTIR test also confirms the presence of methyl and ketone group present in tobacco product extract. And GC-MS

study showed the presence of Acetoin, a chemical compound which produces an alcohol like intoxicating effect.[22]



Based on literature survey, the toxicity of acetoin in rats was investigated and compared to ethyl-alcohol. Acetoin intoxication resembled ethyl-alcohol intoxication leading to respiratory failure. With combined administration, the concentration of acetoin in the blood were additive in effect. It was concluded that acetoin is 1.4 times more intoxicating than ethyl-alcohol.[23] According to above antibacterial activity result; it was observed that the aqueous extract of the tested isolate exhibited good antioxidant activity. The extract and standard exhibited a concentration-dependent increase in their ferric-reducing abilities, indicated by an increase in absorbance with increasing concentration of extract or standard.

**CONCLUSION:**

The tobacco plant, *Nicotiana*, has probably been responsible for more deaths than any other herb. Inspite of the on-going global research about this specie of plant, this study constitutes the first attempt to analyze one of the most commercially available North Indian tobacco products known as Munna Tumbaku. Various analyses involving this tobacco product has led to a brief understanding of its physico-chemical, phytochemical, structural identification and activity of active compound of product aqueous extract. Acetoin, a strong additive in tobacco according to studies have shown to have deep intoxicating actions which could be responsible for health-related diseases caused by tobacco product. Although, not globally known, it has been proven that Acetoin toxicity does not disappear from the blood more rapidly but takes a whole lot of time. Therefore, further studies should be done to understand its biological activity and physiological effect since the effect of Acetoin as a strong intoxicating compound prone to toxicity have not been properly addressed in the field of Medicine.

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