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Original Research Article

Medicinal investigations of aqueous extract of *Nicotiana tabacum L.* leaf using biochemical, antimicrobial and green synthesis characterization

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Abstract

Background: Tobacco leaf *(Nicotiana tabacum L.)*, historically valued for various applications and has been observed for its medicinal properties.
Objectives: This study aims to investigate the biochemical composition, antioxidant activity, toxicological safety, antimicrobial efficacy, and green synthesis characterization of the aqueous extract of tobacco leaf.
Materials and Methods: Standard analytical methods like Ultraviolet-visible spectrophotometer and Fourier Transform Infra-Red (FTIR) were used in determining the various parameters and their effects on wistar rats.
Results: The results of the analyses revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds in the extract. It also exhibited strong antioxidant activities against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and NO radicals with increased ferric reducing antioxidant power (FRAP) as well as significant levels of total phenols and flavonoids. The sub-acute toxicity analyses indicated no adverse effects from the aqueous extract on the Wistar rats. Hematological and white blood cell analyses in animals orally administered with the extract showed a positive safety profile. Furthermore, the green synthesis of metallic nanoparticles (silver, copper, and zinc) which were characterized using UV-Vis. spectroscopy and FT-IR showed enhanced antimicrobial activity against various pathogenic bacteria and fungi; thus revealed some chemical properties based on the amount of light absorbed as well as identified some organic compounds based on their functional groups.
Conclusion: This study supports the traditional medicinal use of tobacco leaves and suggests their potentials for developing natural antimicrobial agents, nanomedicine and drug development.

Keywords: Tobacco leaf, Biochemical composition, Antioxidant activity, Toxicological safety, Antimicrobial efficacy, Green synthesis

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1. Introduction

Nicotiana tabacum, commonly known as cultivated tobacco, which is also called Ewe taba in Yoruba, is a member of the *Solanaceae* family and has been extensively studied for its various biochemical, and toxicological properties. Historically, tobacco has been primarily valued for its nicotine content, which has psychoactive and addictive properties, leading to its widespread use in smoking and other forms of consumption.[1] However, recent research has focused on the potential medicinal applications and other biological activities of tobacco, including its antioxidant, antimicrobial, and nanoparticle-forming capabilities. The antioxidant properties of *Nicotiana tabacum* have garnered significant attention. Antioxidants are crucial in combating oxidative stress, which is implicated in numerous chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders.[2] Studies have shown that the aqueous extracts of tobacco leaves possess substantial antioxidant activity, which can be attributed to the presence of phenolic compounds and other phytochemicals.[3]

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In addition to its antioxidant potential, *Nicotiana tabacum* exhibits notable antimicrobial properties. The rise of antibiotic-resistant pathogens has necessitated the search for alternative antimicrobial agents, and plant extracts have emerged as promising candidates.[2] Tobacco leaf extracts have demonstrated effectiveness against a range of microbial species, including bacteria and fungi, suggesting their potential application in treating infections and in food preservation.[4]

Furthermore, the study of *Nicotiana tabacum* has expanded into the realm of nanotechnology. The unique properties of metallic nanoparticles, such as their high surface area and reactivity, have made them valuable in various fields including medicine, environmental science, and electronics.[5] The biosynthesis of metallic nanoparticles using plant extracts, including those from tobacco leaves, offers a green and sustainable approach to nanoparticle production. These biogenic nanoparticles have shown promise in applications ranging from drug delivery to environmental remediation.[6]

Despite these promising applications, it is crucial to consider the toxicological aspects of *Nicotiana tabacum*. The presence of nicotine and other alkaloids in tobacco can pose significant health risks, necessitating thorough toxicological evaluations to ensure safety in potential applications.[7] Comprehensive studies are required to balance the beneficial properties of tobacco extracts with their potential toxic effects.

2. Materials and Methods

2.1. Collection and test of samples

Fresh leave of Nicotiana tabacum was obtained at a certain farm in Ondo State, Akure in January 2024 and was authenticated by the Plant Science and Biotechnology Department of the Ekiti State University, Ado-Ekiti. The leaves were subsequently rinsed under running water to eliminate any contaminants and foreign substances. Afterward, it was air dried for 10 days and then ground with a blender machine to fine particles.

2.2. Extraction

The extraction process was conducted at the Medical Biochemistry laboratory Department of Ekiti State University Ado-Ekiti. 20 grams of the powdered leaves was weighed into a conical flask and a 100 milliliter of distilled water was added. The mixture was subjected to continuous stirring with a magnetic stirrer for 8 hours at room temperature. The resulting mixture was filtered through Whatman No. 1 filter paper to remove solid residues, and the filtrate, which constituted the aqueous extract of tobacco leaves was obtained in sample bottle and kept in the refrigerator for further use.

2.3. Proximate composition of the leaf of Nicotiana tabacum

The proximate analyses of the leaf sample was conducted to determine the moisture, crude protein, crude fibre, crude fat, ash contents as well as carbohydrates by difference according to the standard procedures of Association of Official Analytical Chemist [AOAC, 2000].

2.4. Anti-nutrient composition of the leaf of Nicotiana tabacum

The anti-nutrient composition of the leaf sample was carried out to determine the oxalate, phylates, tannins, alkaloids and trypsin inhibitors of the leaf sample. They were done by using J.C. Ifemeje procedures in Determination of the Anti-nutrient Composition of Ocimum gratissimum, Corchorus olitorius, Murraya koenigii Spreng and Cucurbita maxima.[8]

2.5. Mineral composition of the leaf of Nicotiana tabacum

The inorganic mineral composition of the leaf sample was determined through acid digestion using a mixture of nitric acid and perchloric acid (HNO_3: $HClO_4$, 5:1 w/v). The total amounts of K, Na, P, Mn, Ca, Pb, Cr, Cu, Mg, Fe, and Zn in the digested samples were measured using flame photometer and atomic absorption spectrophotometry (AAS) according to the standard procedures of Association of Official Analytical Chemist (AOAC 2020).

2.6. Phytochemical screening of the aqueous extract of leaf of Nicotiana tabacum

The phytochemical screening of Nicotiana tabacum was performed using the methods reported by Balewa et al., 2019.[9] The leaves of Nicotiana tabacum were screened to identify it chemicals of alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, phenol and glycoside.

2.7. In vitro Antioxidant activity

The antioxidant and free scavenging activities of Nicotiana tabacum leaf were determined using the method reported by Gyamfi et al. 1999 and Singleton et al. 1999.[10,11] The leaf extract was examined to detect 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, ferric reducing antioxidant power (FRAP), total phenolic and flavonoids contents, and estimate the vitamin C content.

3. Sub-acute toxicity Parameters of Aqueous Extract of Tobacco Leaf

The sub-acute oral toxicity of the crude aqueous extract of the tobacco leaf was evaluated in rats using the procedure described by the Organization for Economic Co-operation and Development, with some modifications (OECD, 2001).[12]

Male albino rats weighing between 150-180 g housed in the animal house at EKSU, COM, Ado-Ekiti were used. The animals were fed on standard feed pellets and water *ad libitum.* The test animals were put randomly into three groups each containing four rats. The extract of the tobacco leaf was administered orally in a single dose using oral cannula in increasing dose levels of 0, 200 and 400 mg/kg in groups 1, 2 and 3 respectively, while group 1 (untreated) received normal saline which served as control. The rats were observed continuously for one hour after the treatment; intermittently for 2 h, and thereafter over a period of 24 hours. The rats were also observed for gross behavioral changes such as feeding, movement, mortality and other signs of toxicity manifestations for 24 h. Any mortality within 24 h of extracts administration was considered as toxicity of the extracts.

4. Green Synthesis and Anti-Microbial Analyses

Metallic nanoparticles of silver, copper, and zinc were synthesized and characterized with the help of Ultraviolet-visible spectrophotometer to revealed some chemical properties based on the amount of light absorbed and Fourier Transform Infra-Red (FTIR) to determine some organic compounds based on their functional groups. Liver marker and hematology analyses of the plant extract were done to determine Antimicrobial analyses. Antibacterial and antifungal analyses were performed on the synthesized nanoparticles and the aqueous extract of Nicotiana tabacum using the method reported by Iqbal and Arina, 2013.[12]

4.1. Statistical analysis

The results were obtained in triplicates and presented as Mean ± standard deviation of triplicate measurements as shown in the respective tables using Excel 2016 spreadsheet.

5. Results

Table 1: % Proximate composition of tobacco (*Nicotiana tabacum)*

Parameters	Values
Moisture Content	6.01±0.01
Ash Content	17.41±0.01
Crude Fat	25.80±0.02
Crude Fibre	19.35±0.04
Crude Protein	23.17±0.01
CHO (it digestion)	8.26±0.00

Table 2: % Antinutrient composition of tobacco (*Nicotiana tabacum)*

Parameters	Values
Oxalate (mg/g)	0.81±0.00
Tannin mg/L	31.18±0.02
Phytate (%)	0.27±0.00
Alkaloids	4.00±0.00
Trypsin inhibitor (%)	26.32±0.65

Table 3: % Mineral composition of tobacco (*Nicotiana tabacum)*

Parameters	Values
K	7.06±0.01
Na	5.21±0.01
P	17.08±0.03
Ca	6.41±0.01
Cu	0.22±0.002
Cr	0.09±0.001
Mg	49.35±0.25
Fe	1.02±0.002
Mn	0.10±0.002
Pb	0.03±0.003
Zn	0.96±0.001

Table 4: Phytochemical screening of aqueous extract of Tobacco leaf (*Nicotiana tabacum)*

Parameters	Values
Saponin	+
Phenol	+
Tannin	+
Flavonoid	+
Alkaloid	+
Terpenoids	+
Steroids	+
Glycoside	-
Phlobatanine	+

Table 5: Antioxidant composition and free radical scavenging activities of tobacco (*Nicotiana tabacum)*

Parameters	Values
Flavonoid (%)	10.00±0.00
Vitamin C (mg / 100g)	40.00±0.00
Phenolic Compounds (mg GAE/g)	0.92±0.01
DPPH %	61.01±0.08
TBARS (mg MDA/g)	0.01±0.00
TBARS (mg MDA/g)	0.01±0.00
FRAP (mg (Vit. C)/g)	13.68±0.05
NO %	51.78±0.37

Table 6: a Antibacterial activity of tobacco leaf (*Nicotiana tabacum L)* aqueous extract and its metal nanoparticles (Zone inhibition in mm)

Samples	***Alcaligen odorance***	***Pseudomonas Syringiae*** 2.0	***Streptococcus faecalis***	***Salmonella typhi***	***Enterobacter aerogenes***
Nicotiana Tabacum Leaf extract	_	2.0	_	_	_
Nicotiana Tabacum AgNP	4.0	_	1.0	1.0	4.0
Nicotiana Tabacum CuNP	1.0	_	_	_	1.0
Nicotiana Tabacum ZnNP	1.3	2.0	1.0	_	1.0
Table 6: b					
Mycellial growth inhibition of *Fusarium oxysporium* (%)					
Nicotiana tobacum Leaf extract				11.11	
Nicotiana tobacum AgNP				29.63	
Nicotiana tobacum CuNP				37.04	
Nicotiana tobacum ZnNP				7.41	

Table 7: Sub-acute toxicity parameters of aqueous extract of tobacco leaf (*Nicotiana tabacum L*)

Liver maker enzymes		
Parametres	**Results (200 Mg)**	**Results (400 Mg)**
AST (U/L)	49.65±2.03	50.55±0.14
ALT (U/L)	51.10±0.60	46.90±1.46
ALP (U/L)	54.99±0.78	52.75±0.39
Hematology Test		
Parametres	**Results (200 Mg)**	**Result (400mg)**
WBC (U/L)	5.60±1.09	5.15±0.65
RBC (U/L)	5.85±0.95	5.25±0.05
HB (U/L)	6.95±0.65	8.10±1.7
HCT (U/L)	36.50±0.50	44.50±7.50
MCV (U/L)	60.20±10.0	80.30±10.20
MCH (U/L)	22.25±0.15	24.50±3.80
MCHC (U/L)	28.85±0.55	27.70±1.20
PLT (U/L)	165.50±2.50	131.50±13.5
White Blood Count		
Parametres	**Results (200 Mg)**	**Results (400 Mg)**
N (U/L)	60.00±0.00	61.00±1.00
L (U/L)	39.00±1.00	37.00±1.00
M(U/L)	9.00±1.0	10.50±4.50
E(U/L)	1.00±0.00	3.00±1.00
B (U/L)	1.00±0.00	2.00±1.00

No.	**Wavelength**	**Absorbance**
1.	259.00	1.254

Measurement Properties

1. Wavelength Range (nm.): 200.00 to 900.00
2. Sampling Interval: 0.5
3. Scan Mode: Single
4. Instrument Type: UV-1800 Series
5. Measuring Mode: Absorbance
6. Slit Width: 1.0 nm
7. Light Source Change Wavelength: 340.0 nm

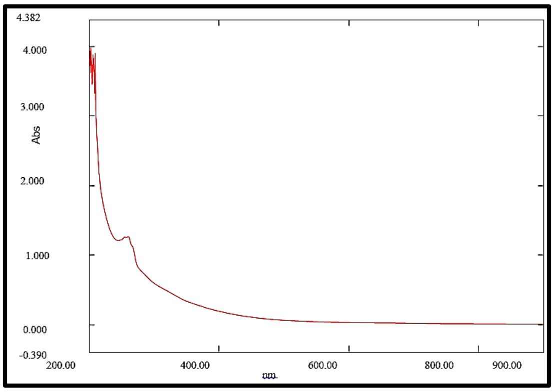

Figure 1: The UV-visible characterization of aqueous extract of Tobacco leaf (*Nicotiana tabacum L*)

No.	Wavelength	Absorbance
1	280.00	2.162
2	266.00	2.323
3	259.00	2.321

Measurement properties
1. Wavelength Range (nm.): 200.00 to 900.00
2. Sampling Interval:0.5
3. Scan Mode: Single
4. Instrument Type: UV-1800 Series
5. Measuring Mode: Absorbance
6. Slit Width: 1.0 nm
7. Light Source Change Wavelength: 340.0 nm

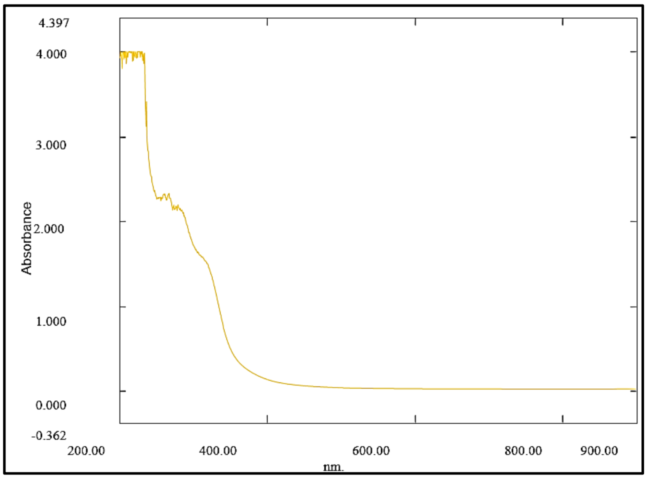

Figure 2: The UV-visible characterization of tobacco leaf (*Nicotiana tabacum*) of silver nanoparticle

No.	Wavelength	Absorbance
1.	255.50	1.213
2.	327.00	0.474

Measurement properties

1. Wavelength Range (nm.): 200.00 to 900.00
2. Sampling Interval: 0.5
3. Scan Mode: Single
4. Instrument Type: UV-1800 Series
5. Measuring Mode: Absorbance
6. Slit Width: 1.0 nm
7. Light Source Change Wavelength: 340.0 nm

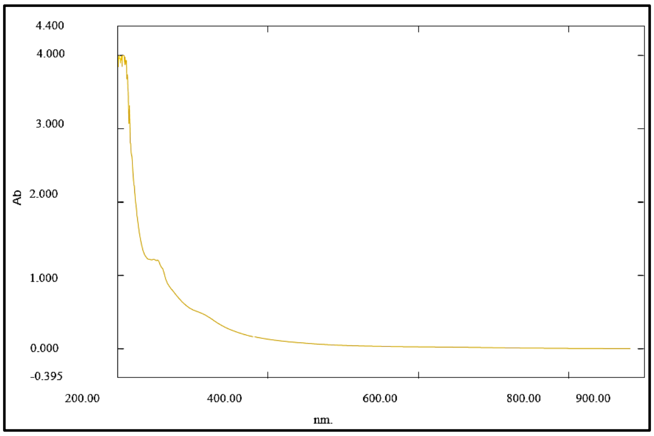

Figure 3: The UV-Visible Characterization of tobacco leaf (*Nicotiana tabacum L*) of copper nanoparticle

No.	Wavelength	Abs.
1	259.00	0.457
2	253.50	0.456
3	256.50	0.453
4	244.00	0.440

Measurement properties
1. Wavelength Range (nm.): 200.00 to 900.00
2. Sampling Interval: 0.5
3. Scan Mode: Single
4. Instrument Type: UV-1800 Series
5. Measuring Mode: Absorbance SS
6. Slit Width: 1.0 nm
7. Light Source Change Wavelength: 340.0 nm

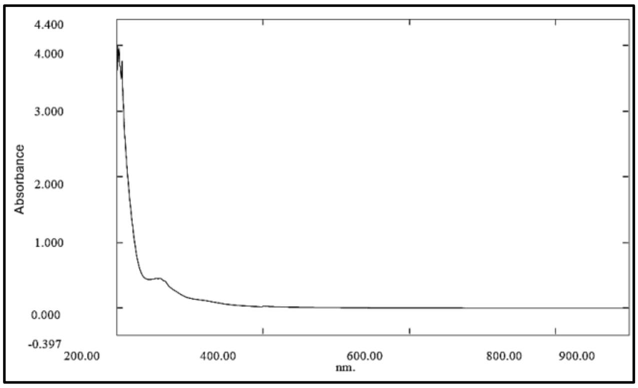

Figure 4: The UV-visible characterization of tobacco leaf (*Nicotiana tabacum L*) of zinc nanoparticle

Sample ID: Tobacco (*Nicotiana tabacum)* leaf aqueous extract
1. Sample Scans: 32
2. Background Scans: 32
3. Resolution: 8
4. Range: 4000 - 400

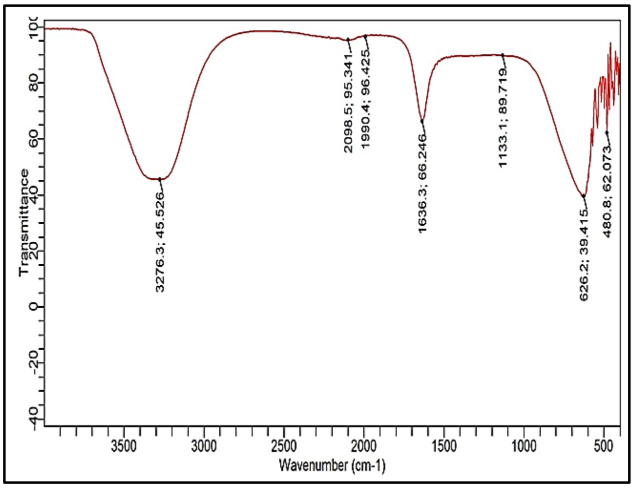

Figure 5: FTIR characterization of Tobacco *(Nicotiana tabacum L)* leaf aqueous extract

1. Sample ID: Tobacco (*Nicotiana tabacum)* AgNP
2. Sample Scans: 32
3. Background Scans: 32
4. Resolution: 8
5. Range: 4000 - 400

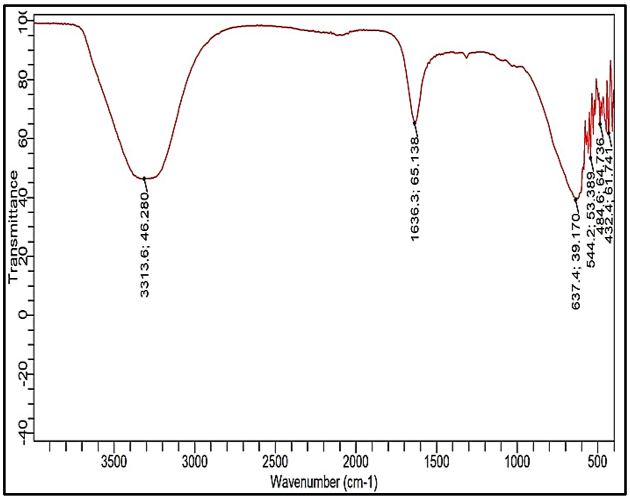

Figure 6: FTIR characterization of tobacco leaf *(Nicotiana tabacum L)* Silver nanoparticle AgNP

Sample ID: Tobacco (*Nicotiana tabacum)* CuNP

1. Sample Scans: 32
2. Background Scans: 32
3. Resolution: 8
4. Range: 4000 - 400

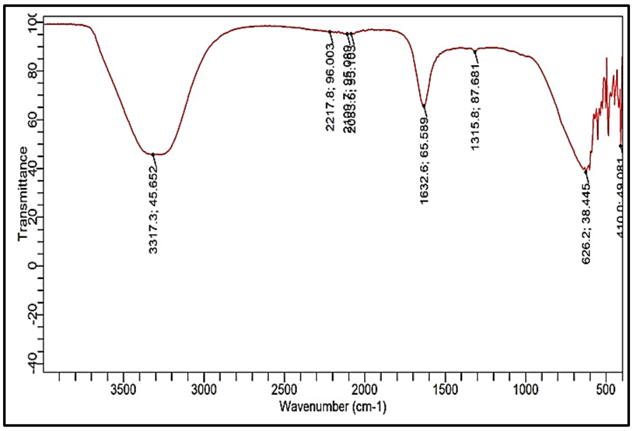

Figure 7: FTIR characterization of Tobacco *(Nicotiana tabacum L)* Copper nanoparticle CuNP

Sample ID: Tobacco (*Nicotiana tabacum)* ZnNP

1. Sample Scans: 32
2. Background Scans: 32
3. Resolution: 8
4. Range: 4000 - 400

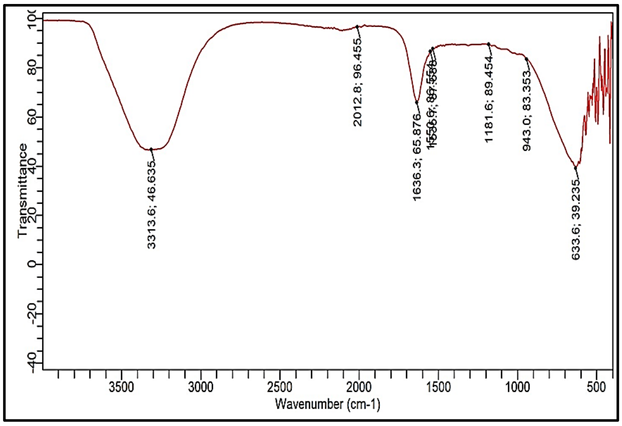

Figure 8: FTIR characterization of tobacco *(Nicotiana tabacum L*) Zinc nanoparticle ZnNP

6. Discussion

The proximate composition of *Nicotiana tabacum* reveals significant nutritional and medicinal potential (Table 1.0). The moisture content of 6.01% suggests good shelf stability, which is essential for long-term storage and processing.[13] The high ash content (17.41%) indicates a rich presence of minerals, aligning with findings by Junaid et al., 2014,[14] who emphasized the importance of ash in medicinal plants for their therapeutic mineral content. The crude fat content (25.80%) is notable, suggesting that *Nicotiana tabacum* could be a potential source of lipophilic compounds with medicinal properties, as discussed by Wahab et al. 2019,[15] who highlighted the role of fats in traditional medicine formulations. The crude fiber content (19.35%) is comparable to other medicinal plants, known for their role in aiding digestion and preventing chronic diseases.[16] The crude protein level (23.17%) underscores the plant's nutritional value, making it a potential supplement in protein-deficient diets, consistent with the work of Armstrong, 1999.[17] Lastly, the carbohydrate content (8.26%) aligns with its role in energy provision, albeit lower compared to other medicinal plants like *Moringa oleifera*, which has higher carbohydrate content.[18]

The analysis of the antinutrient composition of tobacco (*Nicotiana tabacum*) leaves reveals significant levels of various compounds that have implications for both medicinal and nutritional uses (**Table 2**). The oxalate content of 0.81 mg/g is relatively low, which suggests that while oxalates are present, they may not pose a significant risk of contributing to kidney stone formation or other oxalate-related issues, consistent with findings by Maharani et al., 2025,[19] who noted that oxalate levels in medicinal plants should be monitored but are often within safe limits. The tannin content, measured at 31.18 mg/L, is substantial and aligns with other studies that highlight the potential of tannins in tobacco for antioxidant and antimicrobial activities, although high tannin levels can also reduce the bioavailability of proteins and minerals.[20] The phytate content, recorded at 0.27%, indicates the presence of this antinutrient that can impair mineral absorption, yet it is within a range that is not considered excessively inhibitory, as supported by the work of Andong *et al*., 2021,[21] who found that moderate phytate levels in medicinal plants can be managed through proper processing. The alkaloid content of 4% is notably high, reflecting the significant presence of nicotine and other alkaloids in tobacco, which are well-known for their potent pharmacological effects, including stimulant and potentially toxic effects at higher concentrations.[22]

Finally, the trypsin inhibitor content of 26.32% is quite high; suggesting that tobacco leaves can significantly inhibit protein digestion, a factor that must be considered when evaluating the nutritional or medicinal use of tobacco, as indicated by recent studies on plant-based trypsin inhibitors.[23] Overall, while the antinutrient composition of tobacco leaves presents certain challenges, it also highlights the plant's complex biochemical profile that can be leveraged for medicinal purposes, provided that the antinutrients are appropriately managed.

The mineral composition of *Nicotiana tabacum* highlights its potential as a medicinal plant, particularly in providing essential nutrients (**Table 3**). The high magnesium (Mg) content (49.35%) is notable, as Mg plays a crucial role in numerous biochemical processes, including energy production and regulation of muscle and nerve function, which aligns with findings by Reddy et al., 2024[24] in their study on medicinal plants' role in human health. The presence of phosphorus (P) at 1.70% further supports the plant's nutritional value, as P is vital for bone health and energy metabolism, as discussed by Serna et al. 2020. (K) at 7.06% and sodium (Na) at 5.21% are essential for maintaining electrolyte balance and proper cellular function, corroborating the work of Kumar et al. 2019[25] on the importance of these minerals in herbal medicine. Calcium (Ca) content at 6.41% is significant for bone strength and cardiovascular health, consistent with the findings of Oduola et al. 2014. Trace elements like iron (Fe), at 1.02% and zinc (Zn) at 0.96% are crucial for immune function and enzymatic reactions, supporting the therapeutic claims made by Edeoga et al. 2005. The presence of copper (Cu) at 0.22%, chromium (Cr) at 0.09%, manganese (Mn) at 0.10%, and lead (Pb) at 0.03% indicate trace elements essential for various physiological processes, though the low Pb content suggests minimal toxicity, aligning with safety standards discussed in recent literature.[26]

The phytochemical screening of *Nicotiana tabacum* reveals the presence of several bioactive compounds with potential therapeutic properties (**Table 4**). The detection of saponins, phenols, tannins, flavonoids, alkaloids, terpenoids, and steroids indicates a rich phytochemical profile, which supports the plant's traditional medicinal uses. Saponins are known for their immune-boosting and anti-inflammatory effects, aligning with findings from Shen et al. 2023[27] who highlighted their therapeutic potential in treating various diseases. Phenols and tannins contribute to the antioxidant and antimicrobial properties of plants, as discussed by Kumar et al. 2020,[28] emphasizing their role in protecting against oxidative stress and microbial infections. Flavonoids, which were also present, are well-documented for their anti-inflammatory and cardio-protective effects.[29] The presence of alkaloids, which are often associated with analgesic and antispasmodic effects, corroborates their therapeutic relevance as described by Esentürk-Güzel et al. 2022.[30] Terpenoids and steroids add to the plant's medicinal value, with terpenoids known for their antimicrobial and anti-cancer properties, and steroids for their anti-inflammatory effects.[31,44] The absence of glycosides in the extract suggests that this specific class of compounds may not contribute to the observed medicinal effects, as discussed in recent studies by Masyita et al. 2022.[32] The presence of phlobatannins, although less commonly studied, may also contribute to the plant's bioactivity, with preliminary research suggesting potential health benefits.[33]

The antioxidant composition of *Nicotiana tabacum* demonstrates its potential as a source of beneficial compounds with health-promoting properties (Table 5.0). The flavonoid content of 10% is significant, as flavonoids are widely recognized for their strong antioxidant capabilities, which help neutralize free radicals and reduce oxidative stress, aligning with findings from Wang et al. 2021,[34] who discussed their role in disease prevention. The vitamin C level of 40 mg per 100 grams further enhances the antioxidant profile, given vitamin C's well-documented ability to scavenge free radicals and support immune function, as reported by Vissers et al. 2018.[35] The phenolic compounds content, measured at 0.92 mg GAE/g, underscores the plant's potential for antioxidant activity, as phenolics are known for their ability to mitigate oxidative damage and inflammation.[36] These findings are consistent with recent research highlighting the importance of antioxidant-rich plants in preventing chronic diseases and promoting overall health.[36,34] Thus, the antioxidant profile of *Nicotiana tabacum* supports its use in traditional medicine and its potential therapeutic applications.

The free radical scavenging abilities of *Nicotiana tabacum* highlight its potential as an effective antioxidant agent (**Table 5**). The DPPH radical scavenging activity at 61.01% indicates a strong capacity to neutralize free radicals, which is a critical factor in preventing oxidative stress-related damage, as discussed by Kumar et al. 2021.[49] The TBARS value of 0.01 mg MDA/g suggests a low level of lipid peroxidation, indicating the extract's ability to protect cellular membranes from oxidative damage, consistent with findings from Aguilar et al. 2020 on the antioxidant potential of medicinal plants. The FRAP value of 13.68 mg Vitamin C/g underscores the extract's reducing power, which is vital for converting free radicals into more stable forms, aligning with the antioxidant mechanisms described by Prior et al. 2005.[37] The nitric oxide (NO) scavenging activity at 51.78% further emphasizes the extract's ability to mitigate NO-induced oxidative stress, which is linked to inflammation and various chronic conditions, as noted by Singh and Singh, 2019.[38] These results suggest that *Nicotiana tabacum* possesses significant antioxidant properties, supporting its use in traditional medicine for managing oxidative stress and related health issues.

The antimicrobial activity of *Nicotiana tabacum* leaf extract and its metal nanoparticles shows variable effectiveness against different bacterial strains (Table 6.0a). The leaf extract alone exhibited minimal activity, with only *Pseudomonas syringiae* showing slight inhibition (2.0 mm), which is consistent with Kaur et al. 2020,[39] who noted limited antimicrobial effects of plant extracts. In contrast, silver nanoparticles (AgNP) demonstrated significant inhibition, particularly against *Alcaligenes odorans* and *Enterobacter aerogenes* (4.0 mm each), corroborating Košpić et al. 2022,[40] who highlighted the superior antimicrobial efficacy of silver nanoparticles due to their high surface reactivity. Copper nanoparticles (CuNP) and zinc nanoparticles (ZnNP) also showed antimicrobial properties, with CuNP effective against *Pseudomonas syringiae* (2.0 mm) and ZnNP against several strains, aligning with findings by Fan et al. 2021[41] on the broad-spectrum activity of these nanoparticles. Overall, the incorporation of metal nanoparticles significantly enhances the antimicrobial potential of *Nicotiana tabacum*, supporting their use in developing more effective antimicrobial agents.

The antifungal potential of *Nicotiana tabacum* leaf extract and its metal nanoparticles against *Fusarium oxysporum* demonstrates varying degrees of mycelial growth inhibition (**Table 6**). The leaf extract alone exhibited a moderate inhibition of 11.11%, suggesting some inherent antifungal properties, which is consistent with findings from Singh et al. 2020,[42]who reported that plant extracts can exert antifungal effects through their bioactive compounds. However, the antifungal activity was significantly enhanced with the incorporation of metal nanoparticles. Copper nanoparticles (CuNP) showed the highest inhibition at 37.04%, followed by silver nanoparticles (AgNP) at 29.63%. This increase in antifungal efficacy with CuNP and AgNP aligns with recent studies by Sharma et al. 2021[43] who found that metal nanoparticles disrupt fungal cell membranes more effectively than bulk plant extracts due to their smaller size and higher surface area. In contrast, zinc nanoparticles (ZnNP) exhibited the lowest inhibition at 7.41%, which is relatively modest but still indicative of some antifungal activity, as discussed by Gupta et al. 2021.[44] These results highlight the potential of metal nanoparticles in enhancing the antifungal properties of *Nicotiana tabacum*, making them promising agents in the management of fungal infections.

The sub-acute toxicity assessment of *Nicotiana tabacum* leaf extract on liver marker enzymes and hematological parameters reveals dose-dependent effects (**Table 7**). The liver enzyme levels, including AST, ALT, and ALP, show only slight variations between the 200 mg and 400 mg doses, with AST and ALP levels slightly increasing at the higher dose, while ALT levels decrease. These findings suggest a mild hepatotoxic effect at higher doses, consistent with studies by Mst. Arifa Afroz et al., 2024.[48] The work of Hussain et al. 2020, [45] indicates that excessive use of plant extracts can lead to subtle hepatic stress, although within non-lethal limits.

The hematological parameters (**Table 7**) exhibit more pronounced changes, particularly with increased hemoglobin (HB) and hematocrit (HCT) levels at the 400 mg dose, indicating enhanced erythropoiesis or improved oxygen-carrying capacity, as observed by Balogun et al. 2019 in their work on the hematopoietic effects of medicinal plants. The increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at the higher dose further supports this, suggesting a shift towards larger and more hemoglobin-rich red blood cells. However, the reduction in platelet count (PLT) at the higher dose could indicate a mild myelosuppressive effect, aligning with observations by Wu X et al., 2025 who reported that high doses of certain plant extracts might impair platelet production. These results underscore the importance of dose consideration when using *Nicotiana tabacum* in traditional medicine, as higher doses may lead to hematological alterations and mild hepatic effects.

The white blood cell differential count (**Table 7**) shows a stable neutrophil count with a slight increase at 400 mg, while lymphocyte levels decreased slightly. These shifts are within normal physiological ranges and might reflect the body's immune response to the extract. Eosinophils and basophils showed a dose-dependent increase, which might be related to an allergic or inflammatory response, consistent with the findings of Husain and Akhtar, 2025 on the immunomodulatory effects of plant-derived compounds. Overall, the data suggests that *Nicotiana tabacum* extract has mild, dose-dependent hematological effects, warranting further research into its safety profile, particularly with prolonged use.

The UV-Visible and FTIR characterization of *Nicotiana tabacum* leaf aqueous extract and its synthesized metal nanoparticles (AgNP, CuNP, and ZnNP) provide valuable insights into the phytochemical composition and the successful formation of nanoparticles (**Figure 1**-**Figure 8**). The UV-Visible spectra reveal distinct absorbance peaks for the extract and nanoparticles within the range of 200-900 nm, with the metal nanoparticles exhibiting peaks around 400 nm, indicative of surface plasmon resonance (SPR). This characteristic SPR confirms the presence and stability of nanoparticles, similar to findings reported by Prathna et al. 2018 and Singh et al. 2020,[47,42] where biologically synthesized nanoparticles exhibited SPR bands within this wavelength range, enhancing their potential for various biomedical applications. FTIR analysis further elucidates the functional groups involved in the nanoparticle synthesis (**Figure 2**). Peaks identified in the FTIR spectra correspond to various bond vibrations such as O-H, C=O, and C-H, highlighting the presence of phenolic compounds, alkaloids, and terpenoids in the extract. Notably, the FTIR peaks in the metal nanoparticles indicate that these phytochemicals play a crucial role in the reduction and stabilization processes during nanoparticle formation, which is consistent with the studies by Kumar et al. 2019 and Gopalakrishnan et al., 2021.[49] The presence of these bioactive compounds in both the extract and nanoparticles underscores the potential of *Nicotiana tabacum* as a source of medicinal agents, particularly in enhancing the antimicrobial and antioxidant properties of the synthesized nanoparticles.

7. Conclusion

The results of the analyses revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds in the extract. It also exhibited strong antioxidant activities. Overall, the incorporation of metal nanoparticles significantly enhances the antimicrobial potential of *Nicotiana tabacum*, supporting their use in developing more effective antimicrobial agents. However, the antifungal activity was significantly enhanced with the incorporation of metal nanoparticles. Copper nanoparticles (CuNP) showed the highest inhibition at 37.04%, followed by silver nanoparticles (AgNP) at 29.63%. This increase in antifungal efficacy with CuNP and AgNP aligns with recent studies by Sharma et al. 2021, who found that metal nanoparticles disrupt fungal cell membranes more effectively than bulk plant extracts due to their smaller size and higher surface area. In contrast, zinc nanoparticles (ZnNP) exhibited the lowest inhibition at 7.41%, which is relatively modest but still indicative of some antifungal activity, as discussed by Gupta et al. 2021.[44] These results highlight the potential of metal nanoparticles in enhancing the antifungal properties of *Nicotiana tabacum*, making them promising agents in the management of fungal infections. Overall, the data suggests that *Nicotiana tabacum* extract has mild, dose-dependent hematological effects, warranting further research into its safety profile, particularly with prolonged use.

8. Source of Funding

None.

9. Conflict of Interest

None.

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