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Phytochemical Properties and Antimicrobial Susceptibility Testing of "Gegemu" *(Datura stramonium)* Leaf Extract on Bronchitis Causing Organisms

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Abstract

This research was carried out to determine the phytochemical components of "gegemu" (*Datura stramonium*) and its antimicrobial susceptibility on bronchitis-causing organisms: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Haemophilus influenzae*. Phytochemical analysis of aqueous, methanoic and ethanoic extracts of "gegemu" leaf was done. Kirby Bauer Disk diffusion method was used to determine the antimicrobial activities of aqueous, methanoic and ethanoic extracts of "gegemu" leaf. Minimum inhibitory concentration of the extracts against the bacterial isolates was determined. Phytochemical components of the test plant showed the presence of steroid, alkaloids, phenols, tannins, flavonoid, saponin, terpenoid, anthocyanin and anthraquinone. Among the solvents tested, methanol was the best solvent for extracting the bioactive compounds of the "gegemu" leaf. Methanoic extract had bactericidal activity against *Sta. aureus* at 23.5 mg/dl, as against the control, 27.5, 21 mg/dl; 29 mg1dl for *Str. pneumoniae*, 18.5 mg/dl, 26.5 mg/dl for *K. pneumoniae* and 19, 25.5 mg/dl for *H. influenzae*. Conclusively, "gegemu" extract had proven to possess antibacterial activities against the test organisms. The study recommends, among others, that further research be directed toward isolating and characterizing the specific bioactive compounds in Datura stramonium responsible for its antimicrobial properties to better understand their mechanisms of action.

Keywords: Phytochemical, Antimicrobial susceptibility, Aqueous, "Gegemu", Bronchitis

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Introduction

The existence of humans has been connected to plants since life began, relying on them for oxygen, food, shelter, and medicine to survive [1]. Traditional and herbal medicines play vital functions in health services around the world. Roughly 25 % of global prescription drugs come from plants. On the WHO's list of essential medicines, 11 % of 252 drugs are solely plant-based [2]. The use of medicinal herbs in the treatment and prevention of ailments is drawing the attention of scientists worldwide. Scientists are digging deep into herbs, studying their active ingredients, how they work, and potential benefits. Using strict scientific methods like clinical trials and laboratory studies, they are working to back up traditional herbal remedies with solid evidence [3].

Historically, medical care has always played a big role in keeping societies healthy, helping people deal with illnesses and injuries, and nowadays, also preventing diseases and promoting wellness [4]. Mostly, plants have different therapeutic compounds, making them useful as medicines or ingredients to treat different health conditions afflicting man [5]. Research conducted by the World Health Organization indicated that about 80 % of people worldwide rely on traditional healers for plant-based medicines, which come in liquid or dry form [6]. A great number of these Plants are currently used to address various health issues like allergies, arthritis, migraines, fatigue, skin infections, wounds, burns, gut problems, and even cancer backing up the idea that food can be medicine [2]. One of the plants that provides health benefits to humans is *Datura stramonium*.

Gegemu (*Datura stramonium*) is a plant that is found all over the world. [7]. It is an annual plant originating from Asia and Africa. "Gegemu" is mainly present in tropical and temperate regions, belonging to the Solanaceae family. *Datura stramonium* contains tropane alkaloids, including scopolamine, atropine, and hyoscyamine. Because of these important biomedical compounds, it is considered valuable for treating heart disease, dental and skin infections, ulcers, asthma. Bronchitis, leucoderma, piles and sinus infections. *D. stramonium* is known to possess antimicrobial, anticholinergic, anti-inflammatory and anti-fungal functions [8].

Materials and Methods

Collection of "Gegemu" leaves: The leaves of *Datura stramonium* were collected from the botanical garden of Federal College of Education, Abeokuta. The harvested

leaves were packed in plastic containers and transported in those containers to the laboratory for further analysis.
Preparation of leaf extract: Fresh *Datura stramonium* leaves (500 g) were washed thoroughly with water, dried at 35 – 40 °C and then pulverised into a fine powder using an electric grinder. The powdered leaf material was placed in a Soxhlet apparatus with 3,000 mL of 96 % ethanol and extracted by heating at 78 °C for 18 h. The ethanolic and methanolic extracts of *Datura stramonium* leaf powder were concentrated in a rotary evaporator (BUCHI Rota Vapor R-114, Switzerland), yielding a solid residue. This residue was stored at 4 °C until needed, following a modified procedure from [9].
Collection and Maintenance of Test Organisms: The test organisms used in this study were all clinical isolates collected from the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan. The isolates included *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Haemophilus influenzae.* They were collected on a sterile agar slant and incubated at 37 °C for 24 h, after which they were stored as stock cultures on slants in the refrigerator at 4 ºC.

Determination of antimicrobial activity (agar well diffusion method)
The antibacterial effects of aqueous, methanolic, and ethanolic extracts were assessed using the agar well diffusion method.
Bacterial cultures were adjusted to a 0.5 McFarland turbidity standard and inoculated onto a Mueller-Hinton agar (MHA) plate. Each plate was flooded with 1ml of the standardized inoculum, swirled, and the excess liquid was carefully decanted. A sterile cork borer (6 mm diameter) was used to create wells in agar. The extracts were reconstituted in 50 % dimethyl sulfoxide to a concentration of 200 mg/ml. Each well was filled with the appropriate extract, and Ciprofloxacin (200 mg) served as the positive control. Each extract was tested in triplicate. After 1 hour of diffusion at room temperature, plates were incubated at 37 °C for 24 h. Antimicrobial activity was measured as the zone of inhibition (excluding the well diameter) around each well, following a modified method by Otajevwo and Osawaru [10].

Minimum inhibitory concentration (MIC)
The MIC of the extracts against bacterial isolates was determined by the microtube dilution method, following the National Committee for Clinical Laboratory Standards guidelines [11]. Serial two-fold dilutions of the extracts were prepared in an appropriate broth medium in microtubes or microtiter plates. Each dilution was inoculated with a standardized bacterial suspension ($\approx 1 \times 10^6$ CFU/mL). The tubes were incubated at 35–37 °C for 18–24 h. After incubation, the tubes were checked for visible growth. The MIC was recorded as the lowest extract concentration that completely inhibited the growth of the tested bacteria.

Minimum inhibitory concentration (MIC) by tube dilution method:
A 96-well microtiter plate was used for the bioassay, witheach well labelled. In wells 2 to 10 of each row, 100 µL of sterile nutrient broth was added. Then, 100 µL of extract was added to wells 1 and 2, and serial two-fold dilutions were performed from well 2 to well 10, discarding 100 µL from well 10. Next, 100 µL of a 0.5 McFarland bacterial suspension (broth culture) was added to wells 1 through 10. Well 11 received 100 µL of organism suspension and 100 µL of broth (positive control), while well 12 received 100 µL of extract and 100 µL of broth (negative control). Ciprofloxacin was used as the control drug in the experiment [12].

Minimum bactericidal concentration (MBC) assay
From each well that showed no growth (including the MIC well), a subculture was made onto nutrient agar plates. The plates were incubated at 37 °C in ambient air for 24 h. After incubation, the plates were checked for the growth of bacterial/fungal colonies. Lack of growth indicates that the extract has bactericidal potential, while growth indicates bacteriostatic activity.

Results and Discussion

Table 1 displays the phytochemical composition of aqueous, ethanoic, and methanol extracts, showing varying concentrations (mg/100 ml) of compounds like steroids, alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, anthocyanins, and anthraquinones. Tannins are the most abundant in all extracts (4.35, 5.35, and 4.50 mg/100 ml for aqueous, ethanol, and methanol, respectively), indicating potential antiviral, antibacterial, and antiparasitic effects [13]. The methanol extract generally yields higher concentrations of several compounds, including steroids, flavonoids, saponins, terpenoids, and anthraquinone. These phytochemicals are plant secondary metabolites that serve as defense mechanisms against microorganisms and predators [14].

Table 1: Quantitative phytochemical composition of aqueous, ethanoic, and methanoic extracts

Extracts	Phytochemical compounds (mg/100 ml)								
	Steroid	Alkaloids	Phenols	Tannins	Flavonoid	Saponin	Terpenoid	Anthocyanin	Anthraquinone
Aqueous	0.29	0.44	0.33	4.35	0.9	0.73	0.12	0.18	0.14
Ethanol	0.39	0.75	0.47	5.35	1.3	1.3	0.17	0.16	0.30
Methanol	0.54	0.63	0.49	4.50	1.48	1.27	0.23	0.11	0.37

Table 2: Qualitative phytochemical composition of aqueous, ethanoic and methanoic extracts

Extracts	Phytochemical compounds								
	Steroid	Alkaloids	Phenols	Tannins	Flavonoid	Saponin	Terponoid	Anthocyanin	Anthraquinone
Aqueous	+	++	+	+++	+	+	+	+	+
Ethanol	+	++	++	+++	++	++	+	+	+
Methanol	++	++	++	+++	++	++	+	+	+

+ = Present

The aqueous, ethanoic, and methanolic extracts tested positive for various phytochemicals like tannins, alkaloids, phenols, flavonoids, saponin, steroids, etc. highlighting their therapeutic potential as plant secondary metabolites. These compounds have notable properties: alkaloids offer antispasmodic and analgesic effects; terpenoids show antiviral and anticancer activities; phenols and flavonoids exhibit antioxidant and antibacterial properties; and saponins provide anti-inflammatory and plant defense benefits [15].

Table 3 shows the antibacterial activity of leaf extracts against bronchitis-causing bacteria. The methanolic extract had the strongest inhibitory effect, with zones of 23.5 mm (*Sta. aureus*), 21 mm (*Str. pneumoniae*), 18.5 mm (*K. pneumoniae*), and 19 mm (*H. influenzae*) against the aqueous and ethanoic extracts. Ciprofloxacin, the control, showed higher potency with zones of 27.5, 29, 26.5, and 25.5 mm, respectively, demonstrating its effectiveness as a fluoroquinolone antibiotic used in treating urinary tract infections and pneumonia [16].

Table 3: Antibacterial activity of ethanoic, methanoic and aqueous extracts by agar well diffusion test

Extract ID	Organisms/Zone Diameter (mm)			
	Sta. aureus	*Str. pneumoniae*	*K. pneumoniae*	*H. influenzae*
Ethanol	19	19	17	13.5
Methanol	23.5	21	18.5	19
Aqueous	13	14.5	12.5	9
Controls	27. 5	29	26.5	25.5

Control: Ciprofloxacin

Table 4: Minimum inhibitory concentration (MIC) of methanoic, ethanoic, and aqueous extracts

Extract ID	Organisms/ MIC (mg/dl)			
	Sta. aureus	*Str. pneumoniae*	*K. pneumoniae*	*H. influenzae*
Methanol	6.25	3.13	9.38	12.5
Ethanol	6.25	4.69	12.5	18.8
Aqueous	12.5	12.25	18.8	18.8
Control	3.13	3.13	3.13	4.7

Control: Ciprofloxacin

This Table details the minimum inhibitory concentration (MIC) values, indicating the concentration needed to inhibit bacterial growth. Methanolic and ethanolic extracts generally have lower MICs than aqueous extracts, showing greater potency against the tested bacteria. *Str. pneumoniae* is particularly susceptible, likely due to its characteristics as a major nasopharynx pathogen causing various diseases [17].

Table 5: Minimum bactericidal concentration (MBC) of methanoic, ethanoic, and aqueous extracts

Extract ID	Organisms/MBC (mg/dl)			
	Sta. aureus	*Str. pneumoniae*	*K. pneumoniae*	*H. influenzae*
Methanol	9.4	6.25	12.5	18.8
Ethanol	12.5	9.4	18.8	25.0
Aqueous	25.0	25.0	25	50.0
Control	3.13	4.7	3.13	6.25

Control: Ciprofloxacin

Table 5 provides the minimum bactericidal concentration (MBC) values, indicating the concentration needed to kill the bacteria. Methanolic extracts had lower MBCs, showing effectiveness in killing pathogens like *Sta. aureus*, *Str. pneumoniae*, *K. pneumoniae*, and *H. influenzae*, reinforces the discussion point that methanol not only inhibits growth but effectively kill the bacterial and supporting *Datura stramonium's* therapeutic potential for respiratory infections.

This study examined the phytochemical components of *Datura stramonium* leaf extracts and their antibacterial activities against common bronchitis-causing pathogens: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*. The results reveal important insights into the therapeutic potential of "gegemu" as an alternative or complementary treatment for respiratory infections.

Phytochemical screening showed a rich array of bioactive compounds—steroids, alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, anthocyanins, and anthraquinones—in the aqueous, ethanolic, and methanolic extracts. These compounds are well—documented for various pharmacological activities [18, 19]. In particular, tannins and flavonoids are known for antimicrobial and anti-inflammatory effects, which may explain the observed antibacterial action against bronchitis—causing pathogens [19].

Among the solvents, methanol proved to be the most effective in extracting the bioactive phytochemicals, showing higher phytochemical concentrations and stronger antibacterial activity against all tested organisms. This aligns with previous findings in [9], which reported that methanol efficiently solubilizes a

broad range of polar and semi-polar compounds, enhancing extract potency.

The antimicrobial susceptibility results showed that the methanolic extract had strong inhibitory effects on all tested bacteria, with inhibition zones close to the standard antibiotic ciprofloxacin. The MIC values ranged from 3.13 to 18.8 mg/dl, reflecting variable bacterial sensitivities to the extracts. Notably, Streptococcus pneumoniae was the most susceptible organism, requiring the lowest MIC, while *Klebsiella pneumoniae* and *Haemophilus influenzae* showed comparatively higher resistance, necessitating greater extract concentrations for inhibition. The MBC values further confirmed the bactericidal potential of the extracts, with methanol extracts demonstrating lower MBCs than aqueous and ethanolic extracts. This suggests that methanolic extracts not only inhibit bacterial growth but can effectively kill the pathogens, supporting the therapeutic relevance of "gegemu" leaf extracts in treating bronchitis-related infections. The aqueous extracts showed the least activity, which may be attributed to the lower solubility of certain phytochemicals in water and their consequent lower concentration in the extract. These findings highlight the importance of solvent selection in herbal extraction processes to maximize antimicrobial efficacy.

Overall, the study validates traditional claims regarding *Datura stramonium's* medicinal use, especially its role in managing respiratory infections such as bronchitis. The presence of key phytochemicals together with strong antibacterial activity suggests its potential as a natural source for developing new antimicrobial agents, especially given the rise in antibiotic resistance.

Conclusion

This study showed that *Datura stramonium* ("gegemu") leaf extracts possess a wide range of phytochemical compounds with strong antibacterial activity against bronchitis-causing pathogens. Methanolic extracts yielded the highest concentrations of bioactive compounds and exhibited superior antimicrobial efficacy compared to ethanolic and aqueous extracts. The MIC and MBC values indicate that these extracts have both inhibitory and bactericidal effects, especially against *Streptococcus pneumoniae* and *Staphylococcus aureus*, two common bronchitis-causing bacteria.

These results validate the traditional use of *Datura stramonium* in managing respiratory infections and highlight its potential as a source of novel antimicrobial agents. Further research and development are needed to isolate, characterize, and formulate these bioactive compounds for clinical applications. In view of these:

i. Further research should focus on isolating and characterizing the specific bioactive compounds in *Datura stramonium* responsible for its antimicrobial properties to better understand their mechanisms of action.
ii. It is important to develop standardized formulations of the extracts, such as syrups or inhalants, which could be more practical and effective for treating bronchitis.
iii. Investigating the synergistic interactions between *Datura stramonium* extracts and conventional antibiotics could provide novel strategies to enhance therapeutic outcomes and combat antibiotic resistance.

Conflict of interest: The authors declare that there is no any conflict of interest regarding the publication of this research.

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