

Research Article

Proximate, phytochemical and antimicrobial analysis of *Waltheria indica* plant

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Abstract

This study examined the phytochemical constituents, proximate components, and antimicrobial activities of *W. indica* extract. using ethanol as the solvent. The phenol (416.07), flavonoids (26.09), saponins (117.86), tannins (26.45), and alkaloids (20.66) mg/100g were obtained. Also the proximate components obtained are moisture (9.00), ash (4.96), fat (7.84), protein (12.01), fibre (11.02), and carbohydrates (55.17) in percentage (%) respectively. The bacteria isolates were the extract of *W. indica*. The susceptibility test for *E.coli* and *Klebsiella pneumoniae* were (NA) for the extract, but for *Bacillus*, *Salmonella typhi* and *Staphylococcus aureus* the zone of inhibition were 26mm, 19 mm, and 21 mm respectively. The Minimal Inhibitory Concentration (MIC) for *Bacillus* was 120 mg/ml, *Salmonella typhi* (160 mg/ml), and *Staphylococcus aureus* (120 mg/ml). The Minimum Bactericidal Inhibitory Concentration (MBIC) of the extracts was recorded as *Bacillus* (120 mg/ml), *Salmonella typhi* (120 mg/ml), and *Staphi aureus* (120mg/ml) respectively. These results suggest that the studied plant contains phytochemical, proximate components and is very potent for the antimicrobial activities since it shows positive result against selected organisms.

Keywords: Antimicrobia, phytochemical, proximate and *Waltheria indica*

1. Introduction

The increasing global challenge of antimicrobial resistance spurred renewed scientific interest in studying the potential of novel therapeutic agents (WHO, 2020-2024 Report). Plants have served as the cornerstone of traditional medicine systems for millennia, offering a vast repository of bioactive compounds. *Waltheria indica* L. (Syn. *Waltheria americana*), commonly known as sleepy morning, belong to the family malvaceae (formally sterculiaceae) and is widely distributed across tropical and subtropical regions. It is a perennial herb or small shrub extensively used in various folk medicines for treating a multitude of ailments, including inflammatory conditions, respiratory disorders, gastrointestinal issues, wounds, and infections (Burkill, 2000)

The purported medicinal efficacy of *W. indica* is attributed to the complex array of secondary metabolites it synthesizes. A systematic scientific investigation of its phytochemical profile is therefore essential to validate its traditional uses and unlock its pharmacotherapeutic potential (Zongo, *et al.*, 2013). Phytochemical analysis aims to identify and quantify the alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics which are responsible for the plant's biological activities.

Concurrently, proximate analysis provides fundamental data on the plant's nutritional and compositional make up. This includes determining the moisture, ash, crude protein, crude fat, crude fibre, and carbohydrates. Such analysis is crucial not only for assessing the plant's value as a nutraceutical or food supplement but also for standardizing the plant material, which can influence the concentration and extraction efficiency of its active principles.

Given the historical use of *W. indica* in managing infections, a rigorous antimicrobial analysis is of paramount importance. This involves evaluating the plant's extracts against a panel of clinically relevant pathogenic bacteria (e.g. *staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and fungi (e.g. *Candida albicans*). Determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC) helps to understand the antimicrobial activity and provides a scientific basis for its ethnomedicinal application as an anti-infective agent.

Therefore, an integrated study encompassing the phytochemical screening, proximate composition, and antimicrobial efficacy of *W.indica* is justified. This

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multidisciplinary approach will contribute to the pharmacognostic standardization of the plant, offer insights into the chemical basis of its bioactivity, and evaluate its potential as a source of lead compounds.

2. Materials and Methods

2.1. Methodology and Procedures

2.1.1. Plant Sampling

The *W. indica* was obtained in Kontagora along F.U.E. Madara Road. The sample was identified by a Botanist in the Biology Department F.U.E. Kontagora, only healthy leaves were used. The leaves were dried, ground using a machine, and kept for further analysis.

2.1.2. Proximate Composition

Procedure for determination of Moisture Content

2.0g of the powder was weighed into weighed crucible and was put into a Gallenkamp drying oven set at 105°C for 3 h, cooled in desiccators and weighed. Further weighing was repeated until a constant weight was obtained (AOAC, 2000).

$$\% \text{ Moisture} = \frac{(\text{weight of wet sample} - \text{weight of dry sample})}{\text{sample weight}} \quad (1)$$

2.1.3. Ash determination

1.0g of the powder was weighed into a clean crucible and was incinerated in a furnace set at 550 to 600 °C for 3 h. It was removed, cooled in a desiccator and reweighed (Onwuka, 2005).

$$\text{Ash content} = \frac{\text{wt of ash (g)}}{\text{wt of original sample (g)}} \times 100 \quad (2)$$

2.1.4. Crude protein determination

2.0g of the powder was weighed into a digestion flask containing 0.5g of selenium catalyst. 25cm³ of concentrated H₂SO₄ was added and the contents thoroughly mixed. The flask was then heated on a digestion burner for 8 h until the solution was green and clear. The solution was transferred into a 100cm³ volumetric flask and made up to the mark with distilled water. 25cm³ of 2 % boric acid was pipette into a 250cm³ conical flask and 2 drops of mixed indicator (20cm³ of bromocresol green and 4cm³ of methyl red) solutions were added. 10cm³ of the digested sample solution was then introduced into a Kjeldahl flask. The condenser tip of the distillation apparatus containing 15cm³ of 40 % NaOH was dipped into the boric acid contained in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it became bluish green. The distillate was titrated with 0.1M HCl solution until colourless end point was obtained (AOAC, 2000).

$$\% \text{ Total nitrogen} = \frac{(100 \times (\text{VA} - \text{VB}) \times \text{M} \times 0.01401) \times 100}{10\text{W}} \quad (3)$$

Where:

VA= volume (cm³) of HCl used in the sample titration

VB = volume (cm³) of HCl used in the blank titration

M = Molarity of HCl

W = weight of sample (g)

% Crude protein = % nitrogen x 6.25

2.1.5. Crude fibre content determination

2.0g of the defatted powder from crude fat determination was transferred into a 250 cm³ Erlenmeyer flask and 2.5 cm³ of 1.25 % H₂SO₄ was added. The content of the flask was boiled under reflux and digested for 30 min. At the end of the 30min, the content was filtered and subsequently washed with boiling water until the washings were no longer acidic using blue litmus paper. The sample was washed back into the flask with 200 cm³ boiling 1.25 % NaOH solution and boiled for 30 min. It was then filtered and thoroughly washed with boiling water until the washings was no longer alkaline using red litmus paper. The crucible with its content was then dried in an oven at 105 °C overnight and cooled in desiccators and reweighed. The crucible with its content was then ignited in a furnace at 600 °C for 30 min, cooled and weighed. The loss in weight was expressed as a percentage of the initial weight of the sample (AOAC, 2000).

$$\begin{aligned} \% \text{ Crude fibre} &= \frac{(\text{wt. of crucible} + \text{sample before ignition}) - (\text{wt of crucible} + \text{ash}) \times 100}{\text{weight of fresh sample}} \quad (4) \end{aligned}$$

2.1.6. Carbohydrate determination

Total percentage carbohydrate (Nitrogen Free Extract) was determined by the difference method as reported by Amadi *et al*, (2004). This method involves adding the total values of crude protein, crude fat, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat} + \% \text{ fibre}) \quad (5)$$

2.1.7. Procedure for determination of Crude Lipid Content

5.0g of the powder was weighed into the extraction thimble, and about 50 cm³ of petroleum ether (40 - 60 °C) range was added to the extraction flask. A condenser was fixed at the top of the extractor. The flask was fitted into the extraction unit and refluxed to about 60 °C for 6 h. The ether extract was evaporated on an evaporating bath until the

lipid was solvent free. This was dried in an oven at 100 °C for 1 h, cooled in a desiccator and weighed

$$\% \text{Lipid} = \frac{(\text{weight of beaker} + \text{lipid}) - (\text{weight of beaker}) \times 100}{\text{Sample weight}} \quad (6)$$

2.1.8. Phytochemical Screening

The phytochemical analysis of methanol extracts of *W. indica* for tannins, saponins, resin, phlobatanins and alkaloids (Mosses, *et al* 2006 and Harborn ,1998).

2.2. Antimicrobial screening of test organisms

2.2.1. Susceptibility Test

The agar diffusion method was used. The agar (Mueller Hinton Agar) was prepared and dispensed into petri-dish were allowed to gel. The 0.5 mm diameter cork borer was used to bore holes (wells) on the Mueller Hinton Agar.

2.2.2. Standardization of Organisms

The selected organisms were standardized by inoculating 24 h old cultures into 20ml of sterile nutrient broth and incubated for 24 h. 0.2 ml of the previous 24 h culture in broth was taken into another 20 ml sterile nutrient broth and incubated again for 24 h.

2.2.3. Reconstitution of Extract

200 mg/mL was prepared using Dimethyl sulfoxide (DMSO) and (CH₃)₂SO solvent (1.0g dissolved in 5 ml DMSO gives 200 mg/mL.

2.2.4. Screening for Activities

The Petri dishes with hole (wells) were swabbed with 10⁶ CFU using MCFarland Standard. The swab stick was used in the plate and micro pipet to draw the reconstituted wells and this allow for an hour for proper diffusion to take place. The plate was then incubated at 37 °C for 24 h. The active extract produced the zones of inhibition around the wells. The zones of inhibition were recorded in mm diameter.

2.2.4. Minimum Inhibitory Concentration (MIC)

The extracts that were active against the organism were subjected for MIC. The nutrient broth was prepared into test tubes, 2 mL each and 1 ml of the stock solution of the extract was introduced into the test tube and serially diluted and incubated at for 24 hour

3. Results

The results of the phytochemical, proximate and antimicrobial composition of *W. indica* are presented in the tables below:

Table 1: Phytochemical results of *W. Indica*

S/N	Test	Results (mg/100g)
1	Phenol	416.07
2	Flavonoids	26.09
3	Tannins	26.45
4	Saponins	117.86
5	Alkaloids	20.66

Table 2: Proximate results of *W.indica*

S/N	Test	Results (%)
1	Moisture	9.00
2	Ash	4.96
3	Fat	7.84
4	Protein	12.01
5	Fibre	11.02
6	Carbohydrate	55.17

Table 3: Microbial analysis of *W.indica*

S/N	Bacteria	Results (mm)
1	<i>E. coli</i>	NA
2	<i>Bacillus</i>	26
3	<i>Salmonella typhi</i>	19
4	<i>Staphylococcus aureus</i>	21
5	<i>Klebsiella pneumonia</i>	NA

NA= Not Active

Table 4: Minimum Inhibitor Concentration (MIC) of *W. indica*

S/N	Bacteria	Results (mg/ml)
1	<i>Bacillus</i>	120
2	<i>Salmonella typhi</i>	160
3	<i>Staphylococcus aureus</i>	120

Table 5: Minimum Bactericidal Concentration (MBC) of *W. Indica*

S/N	Bacteria	Results (mg/ml)
1	<i>Bacillus</i>	120
2	<i>Salmonella typhi</i>	120
3	<i>Staphylococcus aureus</i>	120

4. Discussion

The *W. indica* contained moisture (9.00 %), ash (7.84 %), protein (12.01 %), fibre (11.02 %), and carbohydrate (55.17 %) respectively. This result is an indication that the plant has nutritional value and can be used as feed, as reported in the literature. The moisture content (less than 10 %) is an indication that the powder of *W. indica* was not contaminated (Wagner and Bladt, 1996). The extract contains phenols (416.07), flavonoids (26.09), saponins (117.86), tannins (26.45), and alkaloids (20.66) in mg/100g respectively shown in table 1. These results corroborate previous studies (Al-Taei, *et al*, 2024).

The susceptibility test showed that *E. coli* and *klebsilla pneumonia* were not active (NA) against the extract but active against *Bacillus Sp*, *salmonella typhi*, and *staphylococcus aureus* at zones of inhibition 26 mm, 19 mm and 21 mm respectively. The extracts were only active against bacteria at zones of inhibition of 120 mg/mL (*basillus*), 160 mg/ml (*salmonella typhi*), and 120 mg/ml (*staphylococcus aureus*) respectively. The Minimum Bactericidal Concentration (MBC) of *W. indica* extract were 120 mm/ml (*basillus*), 120 mm/ml (*salmonella typhi*), and 120 mg/mL (*staphylococcus aureus*) . These results demonstrate that the plant extract possess significant antimicrobial activity (Kim and Sudbery, 2011). It is can seen that *W. indica* shows significant high zones of inhibition values when compared with standard drugs (WHO, 2014).

5. Conclusion

W. indica plant contains significant concentrations of proximate components, phytochemicals, and antimicrobial components which make it nutritious and potentially useful for treating certain ailments. The results of this analysis validate traditional claims of the plant's usefulness in addressing diseases of microbial origin.

Recommendations

1. Further research should be done on the antioxidant of the plant to ascertain it's harmful toxins to humans
2. Other plant parts like the root should be studied to determine the concentrations of the same parameters investigated
3. The plant should be planted in commercial quantity for easy research work
4. The public should be made aware of the benefit of consuming the plant
5. Other organic solvents apart from the Ethanol should be used for the extraction in further research in order to know the most effective solvent to extract the bioactive components of the plant.

Abbreviations

MIC Minimal Inhibitory Concentration

Author Contributions

Mohammed U Manko: Conceptualization, Investigation, Validation, Data Curation, Formal Analysis, Administration, Methodology, Writing - original draft
Rose Ugbede Akpokiere:, Investigation, Writing – Review & Editing

Conflicts of Interest

The author(s) declare that they have no known competing financial interests, professional affiliations or personal relationships that could have appeared to influence the work reported in this paper.

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