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IN VITRO STUDIES ON PHYTOCHEMICAL, ANTIOXIDANT, AND ANTIBACTERIAL PROPERTIES OF ADANSONIA DIGITATA L. FRUIT PULP AND LAWSONIA INERMIS L. LEAVES EXTRACTS.

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Abstract

Background

Plant materials are widely used for their healing power, and many studies have shown that compounds isolated from plants exhibit variable biological properties. The present work was aimed to determine the preliminary phytochemical contents, evaluate the antioxidant capacities, and investigate the antibacterial activities of *Adansonia digitata* L (Bombacaceae) fruit pulp and *Lawsonia inermis* L (Lythraceae) leaves extracts.

Methods

Plant materials were first extracted (separately) by methanolic maceration, then the obtained extracts were utilized for preliminary phytochemical screening tests, in vitro DPPH antioxidant assay, and to determine their antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* using the plate hole diffusion assay.

Results

The preliminary phytochemical screening for *L. inermis* leaf extract revealed the presence of tannins, flavonoids, alkaloids, steroids, and saponins, while *A. Digitata* fruit pulp extract gave positive results for tannins, saponins, cardiac glycosides, terpenes, and flavonoids. Both plant materials exhibited concentration-dependent radical scavenging activity with relatively similar capacities, which is equivalent to the standard (quercetin) at all concentrations (5, 10, 50, 125, and 250 µg/ml). The recorded growth inhibition for *A. digitata* was 19mm against *Klebsiella pneumoniae*, 18mm for *Staphylococcus aureus* and *Proteus mirabilis*, 15mm for *Pseudomonas aeruginosa*, and 14mm against *Escherichia coli*. For *L. inermis*, the inhibition zone was 25mm against *Staphylococcus aureus*, 20mm for *Escherichia coli*, 19mm for *Proteus mirabilis*, 16mm for *Pseudomonas aeruginosa*, and 13mm against *Klebsiella pneumonia*.

Conclusion

The obtained findings could justify the pharmacological properties and may provide the rationale for some ethnomedicinal uses of these plant products.

Recommendation

Further investigations should be performed as they could enhance the medicinal importance and evaluate the traditional values of these plants.

Keywords: *Adansonia digitate, Antibacterial, Antioxidant, Lawsonia inermis, Phytochemical.*
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Introduction

The use of plants for their healing power is an ancient practice, and many people in different countries are still widely using indigenous plants in ethnomedicine, which could be attributed to the plant's ability to produce many aromatic compounds with medicinal usefulness (1). Studies also showed that compounds extracted/isolated from plants were reported to exhibit many biological properties, such as antioxidant and antimicrobial activities, which offer significant values for treating resistant microorganisms (2).

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According to the World Health Organization, the spread of drug-resistance globally leads to many difficulties in treating infections and death, therefore, the need for new antibacterials is urgent, which could lower the cost to the health systems and national economies, and could also affect patients' productivity or their caretakers (3).

Like many African countries, Sudan favors the establishment of many plant species due to variations in climatic zones, which could offer food, pharmaceutical products, and other extracts (4). Among these plants are *Adansonia digitata*, which belongs to the family Bombacaceae and is commonly known as baobab, and *Lawsonia inermis*, which belongs to the family Lythraceae and is commonly known as henna (5). The plant *A. digitata* is a fruit-bearing tree; its fruits and leaves are commonly used for food and medicine, while its bark fibers are used for other domestic purposes (6). In traditional medicine, the baobab parts, especially the fruit pulp, are commonly used in the treatment of diarrhea as an immunostimulant, anti-inflammatory, analgesic, antipyretic, and pesticide (7). Chemically, the baobab is considered rich in antioxidants such as ascorbic acid in the fruit pulp and provitamin A in the leaves (8). Studies also showed that a wide range of chemical substances, such as carbohydrates, amino acids, lipids, vitamins, steroids, flavonoids, and terpenoids, were isolated from *A. digitata* in different parts (7). The other plant, *L. inermis*, which is a small tree, is grown or cultivated in warm and tropical temperature regions, and its leaves are commonly used as dye and coloring matter (9). According to the published data, the henna plant possesses antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties (10). The chemical analysis of henna plant materials indicated the presence of many chemical constituents, such as naphthoquinone derivatives, tannins, coumarins, xanthones, triterpenes, sterols, phenolic derivatives, flavonoids, amino acids, glucose, gallic acid, mannitol, and some minerals (11).

Based on being an indigenous plant that is popularly used for a variety of purposes, along with the previously published data, the present work was aimed to determine the phytochemical contents, evaluate the antioxidant capacities, and investigate the antibacterial activities of *A. digitata* fruit pulp and *L. inermis* leaves extracts, as an initial step which can aid further elucidation of their therapeutic potential and may enhance medicinal uses of these plant products.

Methods

Study Design

The current work was a laboratory experimental study utilized to investigate the phytochemical contents, antioxidant capacities, and antibacterial activities of *A. digitata* fruit pulp and *L. inermis* leaf extracts. It was carried out at the Pharmacognosy and the Pharmaceutical Microbiology laboratories, Faculty of Pharmacy, University of Gezira.

Plant materials and extraction

Fresh leaves of *L. inermis* and fruits of *A. digitata* were obtained from the local market. The plant materials were identified at the Herbarium of the Phytochemistry and Taxonomy Department, Medicinal and Aromatic Plants Institute, National Center for Research, Khartoum, Sudan. The leaves of *L. inermis* (after shed drying at room temperature) and fruits of *A. digitata* (which were crushed to collect the powdered pulp) were ground into powder using a mortar and pestle, then 100 g (dry powder) from each plant material were separately extracted by maceration using methanol (70%) as a solvent system according to the method that adopted by Musab and Elhadi (12).

Preliminary phytochemical screening

From each plant extract, 5 grams were used for preliminary phytochemical screening according to the methods described by Musab and Elhadi (12) for the tests of tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenes, and flavonoids.

In vitro DPPH (1,1-Diphenyl-2-Picrylhydrazyl) antioxidant assay

The antioxidant activities of *A. digitata* fruit pulp and *L. inermis* leaf extracts were estimated according to the procedure described by Bahman et al. (13). One ml of DPPH solution (0.3mM) in ethanol 90% was mixed with 2.5 ml taken from different concentrations (250, 125, 50, 10, and 5µg/ml) of each plant extract. After 30 minutes of incubation in the dark at room temperature, absorbance was measured in a spectrophotometer at 518 nm. The concentrations were prepared in triplicates, and the percentage of the radical scavenging activity (RSA) was calculated by the following equation:

RSA% = [Control – (Sample – Blank)] / Control × 100

Each 2.5 ml taken from the different concentrations of the two plant extracts plus 1 ml of 0.3 mM DPPH solution was considered as a sample, and 1 ml of ethanol plus 2.5 ml of each extract was used as blank, while 1ml of 0.3 mM DPPH solution plus 2.5 ml of ethanol was used as control. Quercetin was diluted to final concentrations of 250, 125, 50, 10, and 5µg/ml in ethanol and used as the reference standard.

In vitro antibacterial activities

The plate hole diffusion assay was used to determine the growth inhibition of bacteria by the extracts of *A. digitata* fruit pulp and *L. inermis* leaves based on the method

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described by Arun et al.. (14). The concentration used for each plant was 100mg/ml. Cefuroxime was used as a standard drug at a concentration of 100mg/ml. The bacterial strains used for this study were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeroginosa*. The mentioned bacterial strains were inoculated into sterile tubes with nutrient broth, and each tube with different species of bacteria was placed in an incubator at 37°C overnight before use. A total of 25ml of molten Muller Hinton agar maintained at 40°C in a water bath was poured into Petri dishes, and then each Petri dish was inoculated with 0.2ml from one of the different bacterial species, mixed well, and allowed to set at room temperature. Using a sterile cork borer 10mm in diameter, four holes per plate were made in the agar containing the bacterial culture. Plant extracts (0.2ml) were poured in three holes, while the fourth one was poured with the standard drug. The plates were kept in an incubator at 37°C overnight, and the mean zone diameter (mm) of three readings for each plant extract against the different bacterial species was then recorded.

Results

Preliminary phytochemical screening

The preliminary phytochemical screening of henna leaves revealed the presence of tannins, flavonoids, alkaloids, steroids, and saponins, while cardiac glycosides and terpenes were not found. For *A. digitata* fruit pulp extract, the qualitative phytochemical analysis gave positive results for tannins, saponins, cardiac glycosides, terpenes, and flavonoids, while it was negative for alkaloids and steroids. The results of the phytochemical screening are shown in Table 1.

Table 1: Preliminary phytochemical screening of *L. inermis* leaves and *A. digitata* fruit pulp extracts

Phytochemical Test	*L. inermis*	*A. digitate*
Tannins	+	+
Alkaloids		
Dragendorff's reagent	-	-
Wagner's reagent	+	-
Mayer's reagent	-	-
Saponins	±	+
Cardiac glycosides	-	±
Steroides	+	-
Terpenes	-	+
Flavonoids	+	+

(+) present, (±) slight, (-) absent.

In vitro DPPH antioxidant assay

Results of the in vitro antioxidant assay using DPPH for *A. digitata* fruit pulp and *L. inermis* leaves extracts using different concentrations (5, 10, 50, 125, and 250 µg/ml) showed high levels of anti-oxidation which is equivalent to the standard (quercetin) at all concentrations of both extracts (Figure 1). The two plant extracts exhibited concentration-dependent radical scavenging activity, and the highest scavenging activity was produced at a concentration of 250 µg/ml which scavenged 90.6% and 89.2% of DPPH for *A. digitata* and *L. inermis* respectively, while the least scavenging activity was produced at a concentration of 5 µg/ml (36.1% and 17.8% for *A. digitata* and *L. inermis* respectively). Both plants showed relatively similar antioxidant activities.

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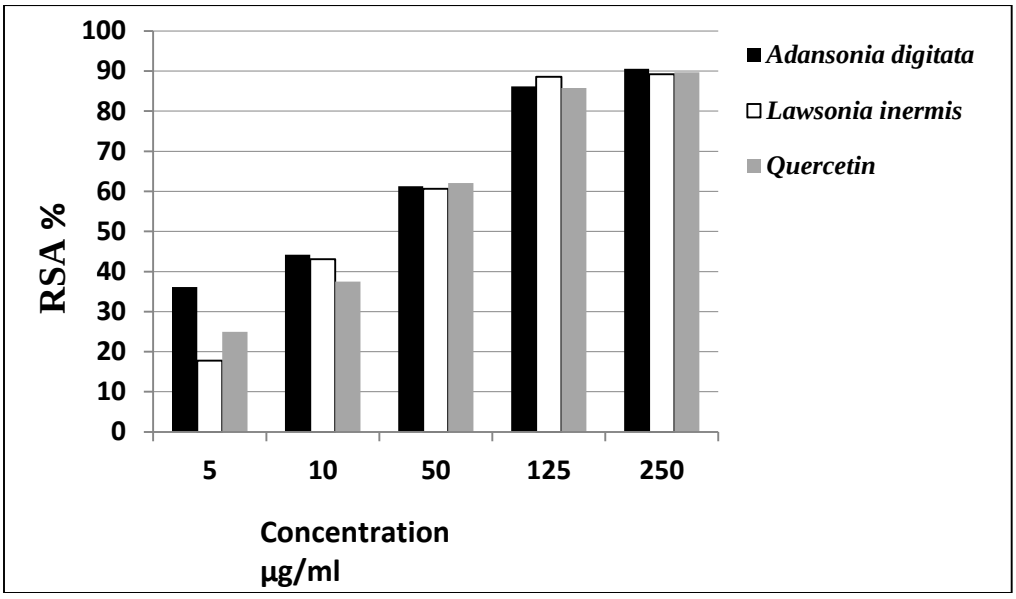

Figure 1: DPPH radical scavenging activities of A. digitata fruit pulp and L. inermis leaf extracts

In vitro antibacterial activities

Results presented in Table 2 indicated that *A. digitata* fruit pulp and *L. inermis* leaf extracts suppressed the growth of the five different tested pathogenic bacteria at varying degrees compared to the used standard drug (cefuroxime). Among the various microorganisms, the extract of *A. digitata* was more active against *Klebsiella pneumoniae* (19mm), followed by 18mm for *Staphylococcus aureus* and *Proteus mirabilis*, 15mm for *Pseudomonas aeruginosa*, and the weakest activity reported against *Escherichia coli* (14mm). For *L. inermis*, the maximum inhibition zone was observed against *Staphylococcus aureus* (25mm), followed by 20mm for *Escherichia coli*, 19mm for *Proteus mirablis*, 16mm for *Pseudomonas aeruginosa*, and the weakest activity reported against *Klebsiella pneumoniae* (13mm

Table 2: Antibacterial activities of A. digitata fruit pulp and L. inermis leaf extracts

Bacteria	Zone of inhibition diameter (mm)		
	***A. digitata* (100mg/ml)**	***L. inermis* (100mg/ml)**	**Cefuroxime (100mg/ml)**
Staphylococcus aureus	18	25	13
Escherichia coli	14	20	21
Klebsiella pneumonia	19	13	24
Proteus mirablis	18	19	22
Pseudomonas aeruginosa	15	16	0

Discussion

Extraction of plant materials could be considered a necessary step for further investigations as it is important to obtain the desired components and obtain a homogenous sample, therefore, proper actions can minimize the loss, distortion, and/or destruction of potential constituents,

furthermore, the chemical constituents could vary between polar, non-polar, and/or thermally labile materials (12). To ensure the suitability of the method and to guarantee that the potential compounds were not affected, methanolic maceration was applied for both plant materials. The findings of the qualitative preliminary phytochemical analysis for *L. inermis* leaf extract were those reported by Upadhyay et al.. and Abdulmoneim (15), (16). For *A. digitata* fruit pulp extract, the presence of tannins, saponins, glycosides, terpenes, and flavonoids was in close agreement with other previous findings (17), (18), (19). However, some differences, such as the absence of alkaloids in the obtained results for *A. digitata* fruit pulp compared to the previously reported data, may be due to the differences in the methods used for extraction or may be due to climatic and environmental factors.

The presence of some phytochemicals that were detected in the current study by preliminary phytochemical screening could justify and provide the rationale for the antioxidant and antibacterial properties of these plant products. Studies showed that plant materials possess antioxidant capacities due to the presence of flavonoids, polyphenols, anthocyanin, coumarins, catechins, isocatechins, and phenolic compounds, which act as free radical scavengers (20). Furthermore, phytochemical constituents such as tannins, flavonoids, and alkaloids have been known to suppress the growth of many microorganisms, and different solvents used for the extraction of plant materials may have the capacity to extract different phytoconstituents with antibacterial activities (21). It has also been reported that flavonoids exhibit high levels of antioxidant activities and are effective scavengers of superoxide anions, peroxynitrite, peroxyl, and hydroxyl radicals, while tannins are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating the microbial proteins (22), (23).

The method of DPPH could represent a convenient, rapid, and easy way to assess the radical scavenging activities and antioxidant capacities of materials (13). The antioxidant activities exerted by *A. digitata* fruit pulp and *L. inermis* leaf extracts may be attributed to the presence of flavonoids that have been revealed by the preliminary photochemical investigation. In addition, it has been reported that *L. Inermis* is a rich plant in phenolic compounds such as phenolic acids, flavonoids, tannins, lignin, and others, which possess antioxidant, anticarcinogenic, and antimutagenic effects as well as antiproliferative potentials (24). Also, studies have confirmed that the baobab fruit pulp can be considered a much more valuable source containing levels of vitamin C (2.8 - 3 g/kg), and the antioxidant capacity of products derived from *the A. digitata* plant was attributed to the high content of vitamin C of the fruit (8).

The determination of the susceptibility of a bacterial pathogen to an antimicrobial agent can be done by the dilution method (permits a quantitative result to be reported) or diffusion method, which permits the report of susceptible or resistant microorganisms compared to the standard drugs (25). Cefuroxime is a member of the second-generation cephalosporins, which is active against many gram-positive and gram-negative bacteria, and it is widely used to treat community-acquired pneumonia because it is active against β-lactamase producing *H. influenzae*, *K. pneumoniae*, and penicillin-resistant pneumococci (26). As reported by Muhammad et al.. (27), plant materials could be considered active against both fungi and bacteria when the zone of inhibition is greater than 6mm. In the current study, the extracts of *A. digitata* fruit pulp and *L. inermis* leaves showed *in vitro* antibacterial activities by inhibiting the growth of all tested microorganisms. The exhibited antibacterial activities against the tested bacterial strains could be due to the presence of tannins and flavonoids that were detected in this study by preliminary phytochemical screening. Furthermore, the recorded growth inhibition for both plant extracts against *Pseudomonas aeruginosa* compared to the standard drug (cefuroxime), which gave 0 mm inhibition zone against the same pathogen, indicated that the plant extracts may possess a prodder antibacterial spectrum than the used standard drug. In addition, it was clear from the obtained results that *L. inermis* leaf extract was more effective than *A. digitata* fruit pulp extract and cefuroxime against *Staphylococcus aureus*, which may correlate with the ethnomedicinal uses of *L. inermis* leaves in the treatment of some skin infections. The obtained data were in close agreement with other previous findings for plants (14), (21), (28), and (29).

Conclusion

The preliminary phytochemical screening revealed the presence of cardiac glycosides, terpenes, tannins, saponins, and flavonoids for *A. digitata* fruit pulp extract and the presence of alkaloids, steroids, tannins, saponins, and flavonoids for *L. inermis* leaf extract. Both plant extracts possess an antioxidant capacity. It was also observed that the extracts of both *A. digitata* fruit pulp and *L. inermis* leaves showed *in vitro* antibacterial activities by inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* at varying degrees.

Limitations of the study

The collection of plant materials should be done in specific seasons during the year.

Recommendations
The obtained findings could justify the pharmacological properties and may provide the rationale for some traditional and ethnomedicinal uses of these plant products, however, further research and investigations should be performed as they could enhance their pharmaceutical/clinical importance and evaluate their traditional values.

List of abbreviations
DPPH: 1,1-Diphenyl-2-Picrylhydrazyl
RSA: Radical Scavenging Activity

Grant information
The author declared that no grants were involved in supporting this work.

Conflicts of interest
I (author) declare that there are no conflicts of interest regarding this work or with anyone.

Data availability
Datasets that support the current research findings are available on request from the corresponding author.

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