AFRICAN JOURNAL OF PHARMACY AND ALTERNATIVE MEDICINE

https://doi.org/10.58460/ajpam.v1i1.29

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ORIGINAL RESEARCH

MJM BIOLABS

Exploring the Synergistic Antimicrobial Potential of Ethanolic Leaf Extracts from *Melia azedarach* and *Solanum incanum* against *Escherichia coli*

Rose Obat[1], Meshack Shiundu[1], Robert Gikaru[1], Collins Bett[1] and Shadrack Ouma[1]

[1]Department of Pharmacology & Pharmacognosy, School of Pharmacy, Kabarak University, P.O. Private Bag Kabarak-20157, Kenya.

[1]Corresponding Author: roseobat@kabarak.ac.ke

Article History

Submitted: 18th June 2023

Accepted: 13th July 2023

Published Online: 16th August 2023

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Abstract

Medicinal plants have been used for maintenance of health for many years. Emerging cases of adverse drug reactions, high cost and antimicrobial resistance using single antimicrobial drug has heightened the need for research into plant sources of antimicrobial activity like *Melia azedarach* and *Solanum incanum*. This research investigated the synergistic antimicrobial activity exhibited by ethanolic leaf extracts obtained from Melia azedarach and Solanum incanum when tested against the pathogenic bacterium Escherichia coli. The study aimed to characterized the presence and composition of phytochemicals in the individual extracts, thereby elucidating their contribution to the observed antimicrobial effects. Additionally, minimum inhibitory concentration (MIC) assays and disc diffusion tests were performed to determine the optimal concentration required to inhibit the growth of Escherichia coli. *Solanum incanum* at concentration of 2g/ml showed an average zone of inhibition of 6.92mm while *Melia azedarach* exhibited a zone of inhibition of 6.97 mm for *Escherichia coli*. The combined extracts showed zone of inhibition of 8.34 mm for *Escherichia coli*. The minimum inhibitory concentration for Solanum incanum was 0.5g/ml while that of *Melia azedarach* was 1.4g/ml for *Escherichia coli*. Leaf extracts of both *Melia azedarach* and *Solanum incanum* had moderate to high levels of tannins, coumarins, flavonoids and anthraquinones. Alkaloids and saponins were in low amounts in both extracts while steroidal glycosides were in moderate amounts in *Melia azedarach* but high in *Solanum incanum*. In conclusion, both plant extracts demonstrated significant antibacterial effect with both plants having a similar potency in terms of zone of inhibition diameter. The combined leaf extract of both plants had higher activity compared to individual plant extracts. We thus recommend that the combined extract can be used as an alternative modality for treating E. coli infections of mild severity without complications.

Keywords: *Solanum incanum, Melia azedarach, Escherichia coli*, Disk diffusion method, MIC, Phytochemicals.

INTRODUCTION

Infectious diseases caused by pathogenic bacteria pose a significant threat to public health worldwide. This threat is more substantial considering the ongoing trend of antibiotic resistance with some of the pathogens mutating to multi-drug resistant strains. This has necessitated the search for alternative antimicrobial strategies of combating infections whereby some aim at rational antibiotic use while others focus on exploring and identifying newer compounds to add to the already diminishing antibiotic pool. Bioactive compounds from plants are rich in chemical diversity and potential therapeutic properties which have made then gain significant attention from the scientific community. Among them is *Melia azedarach* (M. azedarach) and *Solanum incanum* (S. incanum) which have been traditionally recognized for their medicinal properties. Based on literature review, these plants have been shown to have an abundance of phytochemicals including alkaloids, flavonoids, saponins, tannins, and coumarins which possess antimicrobial activity.

Melia azedarach, commonly known as the Chinaberry tree is a deciduous tree native to Australia, Asia and Africa (Batcher, 2015). Various parts of the tree including the leaves have been demonstrated to possess therapeutic activities both from ethnobotanical and in vitro studies (Mlilo & Sibanda, 2022; Khan et al., 2011). Similarly, *Solanum incanum* commonly known as Sodom apple is a shrub native primarily to Africa (Waweru et al., 2017). Its use as medicinal agent especially the leaves has a long history which trails back to traditional medicine (Musyimi et al., 2021). Despite both plants demonstrating antimicrobial activity on an individual basis, exploration of their combined effects remains largely uncharted. The potential synergistic interactions between these two medicinal plants could provide valuable alternatives in managing infectious agents such as *Escherichia coli*.

Escherichia coli is a Gram-negative rod-shaped bacterium that although may be responsible for some of the severe gastrointestinal infections, it is a normal flora of the intestinal tract (Lim et al., 2010). Evidently, the bacteria have several strains of which most are harmless. However, the pathogenic strains have been associated with causing severe urinary tract infections, gastrointestinal infections and life-threatening complications. Currently, multi-drug resistant strains of E. coli have been reported which utilize resistance mechanisms such as synthesizing enzymes that inactivate antibiotics or producing efflux pumps that actively remove antibiotics from the bacterial cell (Uddin et al., 2021). This highlights the need for alternative approaches to combat such infections. Developing alternatives with different active moieties not only adds to the current antibiotic pool but also limits emergence to resistance to such agents as the active moiety works via potentially novel mechanisms. This study therefore aimed at exploring the potential synergistic antimicrobial potential of ethanolic leaf extracts from chinaberry and Sodom apple against E. coli.

METHODOLOGY

Location of the Study

The study was conducted at Kabarak University, utilizing the facilities of the School of Pharmacy laboratory for Pharmacognosy (extraction) and the Pharmaceutical Microbiology lab for evaluation. Fresh *Melia azedarach* leaves were collected from Kabarak University's botanical garden, and *Solanum incanum* leaves were sourced from Rafiki area, Rongai constituency, Nakuru County, Kenya (latitude: -0.2735, longitude: 35.9886). Collected samples were then taken to the Pharmacognosy laboratory for identification by the school botanist, and herbarium specimens were prepared (Herbarium voucher number: KU/HB/2023/001).

Sample Preparation

The collected leaves were washed with distilled water and dried under shade at room temperature for 2 days. After drying, the leaves were pounded using a mortar and pestle to reduce them to a coarse powder. Separate quantities of 150 grams of Solanum incanum powder and 150 grams of Melia azedarach powder were weighed and added to labeled conical flasks, with each flask containing 300 ml of ethanol. The

flasks were then placed on a rotary shaker for three days to facilitate the extraction process. Following extraction, the plant extracts were filtered using Whatman filter paper, and the ethanol solvent was evaporated using a warm water bath to obtain concentrated extracts. Subsequently, paper discs with a diameter of 5 mm were generated by punching dry and clean Whatman filter paper. These discs were soaked in the obtained extracts of different concentrations, as required for the Minimum Inhibitory Concentration (MIC) determination, and allowed to absorb the extract for 24 hours to ensure proper impregnation. This process prepared the test discs for subsequent antimicrobial evaluation.

Media and Inoculum Preparation

Media and inoculum preparation was conducted with strict adherence to standard protocols. Nutrient broth, used for growing the staphylococcal isolates and for determining the Minimum Inhibitory Concentration (MIC), was prepared by dissolving 8.4 grams of nutrient agar in 300ml of distilled water and heated to boiling to ensure complete dissolution. Similarly, Mueller Hinton Agar (MHA) was prepared for culturing bacteria during the disc diffusion method, by dissolving 15.2 grams of MHA in 400ml of distilled water and heating it to boil for complete dissolution. Both media were subjected to sterilization through autoclaving at 121°C for 15 minutes, followed by cooling to 45°C. Media sterility checks were performed by incubating samples of each medium alone at 37°C for 24 hours to verify their ability to support growth. Additionally, media viability checks were carried out by inoculating samples of each medium with bacteria to confirm their efficacy in supporting bacterial growth. *Escherichia coli* isolates were sourced from the reservoir stored in the Pharmaceutical Microbiology Laboratory, School of Pharmacy, Kabarak University. The bacteria were grown in nutrient broth and incubated at 37°C for 24 hours, following the CLSI (2022) guidelines.

Phytochemical Analysis

Seven classes of phytochemicals were tested including: tannins, flavonoids coumarins anthraquinones, saponins, phytosterols and alkaloids (Mutungi et al. 2023; Ahmed and Sulaiman 2018).

Table 1:

List of Phytochemical Classes and the Tests Used to Evaluate their Presence

Phytochemical class	Detection test	Procedure
Tannins	Iron chloride test	The ethanolic extract was dissolved in distilled water then filtered. 3 drops of ferric chloride were added. Formation of grey color indicates presence of tannins.
	Lead acetate test	To the ethanolic extracts, 3 drops of lead acetate were added. Formation of white buffy precipitate indicates presence of tannins.
	Iodine test	Dilute iodine was added to the ethanolic extracts. Appearance of transient red colour indicates presence of tannins.
	Acetic acid test	A few drops of Acetic acid were added to small amounts of the ethanolic plant extracts and the colour change observed. A green or blue color indicates presence of tannins.

Phytochemical class	Detection test	Procedure
Flavonoids	Alkaline test	Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless (dilute acid is used) indicates the presence of flavonoid.
	Shinoda test	A few fragments of magnesium turnings and concentrated sulphuric acid were added to the ethanol extract. Appearance of red to pink color after few minutes indicates presence of flavonoids.
	Lead acetate test	Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.
	Ammonia test	Dilute ammonia was added to a portion of the extract then concentrated sulphuric acid added. Formation of a yellow brown colour.
Coumarins	Iron chloride test	Few drops of FeCl3 solution was added to the ethanolic extracts. Formation of dark green colour indicates presence of coumarins.
Anthraquinones	Bontragers test	The extracts were boiled with dilute sulphuric acid and chloroform added to the filtrate and shaken well. The lower layer was obtained then few drops of ammonia added. The lower ammoniacal layer takes pink color.
	Modified Bontragers test	3 drops of Ferric chloride were added to the sulphuric acid extract and boiled for 2 minutes and then filtered and chloroform added to the filtrate and shaken well. The lower layer was obtained then few drops of ammonia added. The lower ammoniacal layer takes pink color.
Saponins	Froth test	5m of extracts was shaken for about 1 minute, and then allowed to stand for 10 minutes. Formation of persistent froth indicates presence of Saponins
Phytosterols	Salkwoski test	Extract treated with chloroform and filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
	Lieberman's test	Extracts were treated with chloroform and filtered. The filtrates are treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid is added. The formation of brown ring at the junction indicates the presence of phytosterols.
Alkaloids	Mayer's test	Extracts were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.
	Wagner's test	Extracts were treated with Wagner's reagent (iodine in potassium iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.
	Dragendorff test	Extracts were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of a red precipitate indicates the presence of alkaloids
	Hager's test	Extracts were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colour indicates the presence of alkaloids

Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentrations (MICs) were determined through serial dilution of the plant extracts at four different concentrations. Fourteen grams of Melia azedarach leaf extract and 10 grams of *Solanum incanum* leaf extract was separately diluted in 5 ml of dimethyl sulfoxide (DMSO) in the first pair of test tubes. Subsequently, 2 ml of DMSO was added to the remaining three test tubes. In a stepwise manner, 1 ml of the mixture from the first test tube was transferred to the second test tube and thoroughly mixed. Then, 1 ml from the second test tube was drawn and transferred to the third test tube, and the same process was repeated for the fourth test tube. This resulted in the preparation of four different concentrations for Solanum incanum (2g/ml, 1g/ml, 0.5g/ml, 0.25g/ml) and Melia azedarach (2.8g/ml, 1.4g/ml, 0.7g/ml, 0.35g/ml) extracts, respectively. The serial dilutions allowed for a range of concentrations to be tested, enabling accurate determination of the MIC values for each extract.

Disc Diffusion Method

The prepared Mueller Hinton Agar (MHA) medium was aseptically added to sterile culture plates and allowed to solidify. Using a sterile wire loop, bacterial isolates obtained from the staphylococcal inoculum were streaked onto the MHA culture medium in a zigzag pattern. Subsequently, paper discs impregnated with the plant extracts at different concentrations were carefully placed on top of the streaked culture media. To ensure sufficient spacing, appropriate distances were maintained between the discs themselves and between the discs and the walls of the petri dishes. As positive and negative controls, Gentamicin and dimethyl sulfoxide (DMSO) were used, respectively. Following preparation, the cultures were incubated at 37°C for 24 hours. After the incubation period, the diameter of the zone of inhibition around each disc was measured using a Vernier caliper and the values were tabulated for further analysis. This methodology allowed for accurate assessment of the antimicrobial activity of the plant extracts against the test organism, *Staphylococcus aureus*.

Ethical Considerations

Ethical considerations were meticulously addressed throughout the course of this study. Approval was obtained from the School of Pharmacy, ensuring adherence to institutional guidelines and regulations. Additionally, the study received ethical clearance from the Kabarak University Research Ethics Committee (KUREC), which ensures the protection of human subjects and upholds ethical standards in research. Furthermore, the National Commission for Science, Technology, and Innovation (NACOSTI) granted the necessary research permit, affirming compliance with national regulations and guidelines for conducting research involving human subjects or biological samples. The researchers followed rigorous ethical protocols to safeguard the rights and well-being of study participants, ensure informed consent, and maintain data confidentiality. Ethical considerations extended to the handling of plant materials, ensuring sustainable collection practices and preservation of the local biodiversity.

Data Analysis

Data analysis was performed SPSS, and the results were presented descriptively and inferentially. The antimicrobial activity of *Melia azedarach* and *Solanum incanum* extracts against *Escherichia coli* was assessed by measuring the zone of inhibition around each disc. The obtained data were subjected to statistical tests, such as analysis of variance (ANOVA) and post-hoc tests, to determine significant differences between the various concentrations of the plant extracts. The minimum inhibitory concentration (MIC) values were determined through serial dilution experiments. Additionally, the phytochemical composition of the extracts was analyzed using descriptive statistics. The results were presented in tables, graphs, and figures.

RESULTS

Extraction Results

Table 2 below shows the extraction yield results of the two plant specimens. A total of 14 grams and 10 grams were extracted from *Melia azedarach* and *Solanum incanum* plant species respectively corresponding to a percentage yield of 9.3% and 6.7%.

Table 2:

Mass of Ethanolic Leaf Extract of Melia azedarach and Solanum incanum Obtained from the Extraction Process

Plant extract	Extract mass	Percentage Yield
Melia azedarach	14 grams	9.3%
Solanum incanum	10 grams	6.7%

Phytochemical Analysis

Solanum incanum and *Melia azedarach* leaves extracts had the following phytochemicals Anthraquinones, saponins, flavonoids, coumarins, steroidal glycosides, tannins and traces of alkaloids, which could have contributed to the antimicrobial activities of the plant extracts.

Table 3:

Phytochemical Analysis of Ethanol Leaf Extracts of Melia azedarach and Solanum incanum

Test	Observation	Inference
ANTHRAQUINONES		
Bontragers test *Solanum incanum* -Pink colour	*Melia azedarach*-Formation of pink color	+++ (high presence)
	+++ (high presence)	
Modified Bontragers test *Solanum incanum* -Pink colour	*Melia azedarach* -Formation of pink colour	++ (moderate presence)
	+ (low presence)	
SAPONINS		
Froth test *Solanum incanum* -Formation of persistent froth	*Melia azedarach*-Formation of persistent froth	+ (low presence)
	+ (low presence)	
FLAVONOIDS		
Alkaline test	*Melia azedarach*-Brown colour which disappears on adding H2SO4	+++ (high presence)
	Solanum incanum -Brown colour which disappears on adding H2SO4	+++ (high presence)
Shinoda test *Solanum incanum* -Formation of red colour	*Melia azedarach*-Formation of red pink colour	+ (low presence)
	+++ (high presence)	

Test	Observation	Inference
lead acetate *Solanum incanum* -Formation of yellow colour	*Melia azedarach*-Formation of yellow colour	+++ (high presence)
	+++ (high presence)	
Ammonia test *Solanum incanum* -Formation of yellow brown colour	*Melia azedarach*-Formation of yellow brown colour	+++ (high presence)
	+++ (high presence)	
COUMARINS		
$FeCl_3$ test	*Melia azedarach*-Formation of dark green colour	+++ (high presence)
	Solanum incanum -Formation of dark green colour	+++ (high presence)
STEROIDAL GLYCOSIDES		
Salkwoski test *Solanum incanum* -Formation of brown ring	*Melia azedarach*-Formation yellowish fluorescence	++ (moderate presence)
	+++ (high presence)	
Liebermans test *Solanum incanum* -Formation of brown layer	*Melia azedarach*-Formation of brown colour at lower ring	++ (moderate presence)
	+++ (high presence)	
TANNINS		
$FeCl_3$ test *Solanum incanum* -Formation of green colour	*Melia azedarach*-Formation of green colour	+++ (high presence)
	+++ (high presence)	
Lead acetate *Solanum incanum* -White brown buffy precipitate	*Melia azedarach*-White brown buffy precipitate	+++ (high presence)
	+++ (high presence)	
Iodine test *Solanum incanum* -No yellow colour	*Melia azedarach*-Formation of yellow colour	+++ (high presence)
	___ (no presence)	
ALKALOIDS		
Mayers test *Solanum incanum* -Slight yellow ppt	*Melia azedarach*-Slight yellow ppt	+ (low presence)
	+ (low presence)	
Wagners test *Solanum incanum* -Slight brown ppt	*Melia azedarach*-Slight brown ppt	+ (low presence)
	+ (low presence)	
Hagers test *Solanum incanum* -Slight yellow color	*Melia azedarach*-Slight yellow colour	+ (low presence)
	+ (low presence)	
Dragendorfs test *Solanum incanum* -Slight red ppt	*Melia azedarach*-Slight red ppt	+ (low presence)
	+ (low presence)	

Minimum Inhibitory Concentration Results

The MIC of *Solanum incanum* extract against *Escherichia coli* was 0.5g/ml while for *Melia azedarach* extract against was 1.4g/ml. For the combined extracts, the MIC was observed at 25%.

Table 4:

Minimum Inhibitory concentration of Ethanol Leaf Extracts of Melia azedarach and Solanum incanum Against Escherichia coli

Solanum incanum		*Melia azedarach*		*Combined plant extracts*	
Concentration (g/ml)	observation	Concentration (g/ml)	Observation	Concentration (g/ml)	observation
2	Clear	2.8	Clear	100	Clear
1	Clear	1.4	Clear (MIC)	50	Clear
0.5	Clear (MIC)	0.7	Turbid	25	Clear (MIC)
0.25	Turbid	0.35	Turbid	12.5	Turbid

Disc Diffusion Results

Solanum incanum ethanolic extract showed activity against *Escherichia coli*. Highest and lowest zone of inhibition diameter for Solanum incanum was observed at 2g/ml (6.92mm) and 0.25g/ml (5.3mm) while for Melia azedarach was observed at 2.8g/ml (6.97mm) and 0.35g/ml (5.21mm) respectively. Highest zones of inhibition for the combined extract was 8.34mm at 100% extract concentration while lowest diameter was found at 12.5% concentration (6.36mm). The activity of the combined extracts was more compared to individual antibacterial activity of each plant extract.

Table 5:

In-Vitro Antimicrobial Activity of Ethanol Leaf Extracts of Melia azedarach and Solanum incanum Against Escherichia coli

Solanum incanum		*Melia azedarach*		Combined plant extracts	
Concentration (g/ml)	Average zone of inhibition (mm)	Concentration (g/ml)	Average zone of inhibition (mm)	Concentration (g/ml)	Average zone of inhibition (mm)
2	6.92	2.8	6.97	100	8.34
1	6.71	1.4	6.79	50	7.6
0.5	6.30	0.7	5.64	25	6.94
0.25	5.30	0.35	5.21	12.5	6.36
Positive control (gentamicin)		21.39 mm			
Negative control (DMSO)		-			

DISCUSSION

This study aimed at assessing the synergistic antimicrobial potential of ethanolic leaf extracts from Melia azedarach and Solanum incanum against the bacterium Escherichia coli. This was achieved by determining the yield potential, screening for various phytochemicals and conducting disk diffusion test and determination of minimum inhibitory concertation. Out of 150 grams of crude powder for *Melia azedarach* and 150 grams of *Solanum incanum*, a percent ethanolic yield of 9.3% and 6.7% respectively was obtained. Phytochemical screening of both extracts showed an abundance of anthraquinones, tannins, flavonoids, and coumarins. Alkaloids and saponins were in low amounts while steroidal glycosides were higher in *Solanum incanum* extract. Alhadrami et al. (2022) showed that anthraquinones have antimicrobial activity against multidrug-resistant E. coli. Additionally, studies by

Monte et al. (2014) and Adnan (2014) also report that that other phytochemicals such as tannins, flavonoids, and coumarins also possess antimicrobial activity. Correspondingly, this would suggest that the observed antibacterial activity of both plant extracts was due to the plant phytochemicals that were found to be present in abundance from the screening exercise.

To note however, in most cases, the presence of a phytochemical does not directly translate to the extract having activity against the microorganism. A minimum concentration termed minimum inhibitory concentration (MIC) is to be achieved for a plant's extract to show antimicrobial effect (Andrews, 2001). Evidently, in this experiment, the MIC for *Solanum incanum*, *Melia azedarach* and combined extract was 0.5g/ml, 1.4g/ml and 50% respectively. Therefore, in the disk diffusion test, while all concentrations showed some activity against E. coli, much emphasis was on concentrations above the MIC level. Evidently, an increment in the dose of the extract was accompanied with a correspondingly increase in antibacterial activity against E. coli as shown in table 5. This means that while the antibacterial activity of the extract is low compared to gentamicin (positive control), if sufficiently larger enough concentration of the plant extract is use, it might equal or even surpass that of the conventional drug. Moreover, since the synergistic effects are more than that of the individual drug, at the equipotency level to gentamicin, lesser amount of the extract would be required compared when used individually.

CONCLUSION

In conclusion, the percentage extract yield for *Solanum incanum* and *Melia azedarach* was 6.7% and 9.3% respectively. Leaf extracts of both *Solanum incanum* and *Melia azedarach* had an abundance of anthraquinones, tannins, flavonoids, and coumarins but low in alkaloids and saponins. Further, the MIC level for *Solanum incanum* leaf extract, *Melia azedarach* leaf extract and the combined leaf extract was 0.5g/ml, 1.4g/ml and 50% respectively. All the four concentrations of both extracts exhibited antimicrobial activity. The combined activity of the leaf extracts was higher compared to the antibacterial activity of individual plant extracts for both plant samples.

RECOMMENDATIONS

We recommend that combined leaf extract of *Solanum incanum* and *Melia azedarach* can be used as an alternative in the management of E. coli infections especially those of mild severity without complications. We also recommend that further studies be done to identify, isolate and purify individual bioactive components responsible for the antibacterial activity to reduce final consumer volume and minimize side effects.

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