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Original Research Article

Determination of the antibacterial activity of the phytochemicals from Melia azedarach plant extract

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Abstract

Commercial medicine has become very expensive and this has resulted in the integration of traditional medicine into commercial drugs. This integration has led scientific researchers to work on different herbs that are used for medicinal purposes in order to identify and isolate the phytochemicals responsible for their therapeutic effects. This study focuses on determination of the antibacterial activity of the phytochemicals from the leaves of the *Melia azedarach* plant. *Melia azedarach* has been used intensively in Zimbabwe for many therapeutic purposes such as anti-diarrhoeal, deobstruent, cathartic, and emutic. The aim of this study was to identify and isolate phytochemicals with antibacterial activity against *Staphylococcus aureus*, from the leaves of *Melia azedarach*. Phytochemicals were qualitatively and quantitatively determined using different specific methods for each phytochemical. The isolated phytochemicals were then investigated for their antibacterial activity against *Staphylococcus aureus* using the disc diffusion and broth dilution assays. The results showed that the phytochemicals that were present in the leaf extract are alkaloids, flavonoids, tannins, saponins, and cardiac glycosides. The alkaloids had the most potent activity against *S. aureus* with minimum inhibitory concentrations of 50µg/ml. Thus, alkaloids from *Melia azedarach* leaves may be used as a template for the development of antibacterial agents against *Staphylococcus aureus*.

Keywords: Melia azedarach, Staphylococcus aureus, phytochemicals, antibacterial activity

INTRODUCTION

The consumption of herbal medicines is increasing throughout the world as an alternative treatment for alleviating several problems including heart diseases, diabetes, high blood pressure and even certain types of cancer. Traditional herbal medicines are naturally occurring plant-derived substances that are used to treat illnesses within local or regional healing practices [1]. The intensive use of these traditional medicines has resulted in their rational integration into modern medical practices, including cancer therapy [2].

This current study focuses on the identification and isolation of phytochemicals with antibacterial activity against *Staphylococcus aureus*, from the leaves of *Melia azedarach*. *Melia azedarach* is a plant that has been used massively in India and other parts of the world for medicinal purposes. *Melia azedarach* is used as an Ayurveda medicine in India and Unani medicine in Arab countries, as anti-oxidative, analgesic, anti-inflammatory, insectidal, rodenticidal, anti-diarrhoeal, deobstruent, diuretic, antidiabetic, cathartic, emutic, anti-rheumatic, and antihypertensive [3]. *Melia azedarach* is native in south Asia and has been naturalized in Zimbabwe and most parts of the world including America. In Zimbabwe, it is known as *mukina* or *musiringa*, but in English, it is known as Chinaberry tree or Indian lilac. The tree belongs to the mahogany family *Meliaceae* [4].

Staphylococcus aureus is a major cause of hospital-acquired infection of surgical wounds and infections associated with indwelling medical devices. It causes serious infections, such as, pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, and deep-seated infections. *Staphylococcus aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by the release of super-antigens in the bloodstream [5].

The fact that *S. aureus* causes several medical conditions, some plants that are considered to be herbs have been used to counter or rather treat these conditions as the bacteria has grown to be resistant to some antibiotics. Thus, in this study, tests for the antibacterial activity against *S. aureus* were carried out using phytochemicals isolated from *Melia azedarach* plant leaves.

METHODOLOGY

Collection of plant leaves

Mature fresh green leaves of M. azedarach were collected at the National Archives of Zimbabwe (38.8925° N, 77.0229° W), in June 2021. The plant was identified and authenticated by Mr Chistopher Chapano, a botanist at the Botanical gardens in Harare, Zimbabwe. The leaves were washed, separated from stems, and left to dry in the open air on the workbench. After about a week, the dried leaves were crushed using mortar and pestle to a fine powder to increase surface area for extraction. The powdered sample was stored in an airtight jar to prevent moisture.

Qualitative Analysis

Chemical constituents from Melia azedarach leaves were determined using the standard procedures with some modifications [6,7,8].

Quantitative Analysis

Flavonoids were quantified using the method by Bohm and Kocipai-Abyazan [9]. About 10 g of powdered sample were extracted twice with 80 % methanol at room temperature. The mixture was then filtered using a Whatman Filter paper. The filtrate was heated over a water bath to dry and evaporate to a constant weight. The obtained product was measured and recorded as flavonoids. Tannin's determination was done using methods by Van-Burden and Robinson with modifications [10]. About 2 g of powdered sample were added to a 500 ml flask with 50 ml of distilled water. The mixture was put in a shaker for 1 hour. The solution was then filtered into a 50 ml volumetric flask and filled to the mark. To 5 ml of the solution, 2 ml of 0.1M FeCl2 in 0.1N HCl and 0.008M potassium ferrocyanide were added. Absorbance at 650 nm was measured within 10 minutes. The absorbance obtained was used to calculate the amount of tannins in the sample using the standard curve. Saponins were quantified using the method by Obadoni and Ochuko [11]. About 2.5 g of the powdered sample were added to a volumetric flask with 20 % ethanol. The mixture was put in a shaker for 30 minutes. The solution was then heated over a water bath for 4 hours at 55 ºC. The solution was then filtered and the residue was extracted with 20 % ethanol for 2 hours. The combined extracts were reduced to 40 ml over a water bath at 90 ºC. The concentrate was then extracted twice with 20 ml of diethyl ether. The ether layer was discarded and the aqueous layer was retained. A volume of 60 ml of n-butanol was added and the extract was washed with 10 ml of 5 % aqueous NaCl. The remaining solution was heated over a water bath and after evaporation; the sample was dried in an oven at 40 ºC to a constant weight. Alkaloids were quantified by the Harborne method [8]. About 5 g of the powdered sample was added to a 200 ml solution of 10 % Acetic acid in ethanol. The mixture was covered and allowed to stand for 4 hours. The mixture was filtered and the filtrate was concentrated to 25 % of the initial volume. Concentrated ammonium hydroxide was added dropwise to extract until precipitation was complete. The whole solution was allowed to settle, then filtered and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered again. The residue was dried and weighed and expressed as alkaloids.

Antibacterial Activity Tests

Disc diffusion assay

Staphylococcus aureus was resuscitated in nutrient broth and cultured overnight at 37 ºC and 120 rpm. Nutrient agar was then prepared, autoclaved at 121 ºC for 15 minutes and then allowed to cool. The overnight culture of Staphylococcus aureus was then inoculated into agar to a final concentration of 1 x 10-6 cfu/ml. The agar was poured into plates and left to solidify. Discs of 6 mm in diameter, loaded with different extracted phytochemicals with equal concentration were placed on top of agar in plates. The plates were then put into the refrigerator for about 2 hours for diffusion to take place. After 2 hours, the plates were incubated at 37 ºC overnight.

Broth Dilution assay

Isolated phytochemicals from Melia azedarach (100 µg/ml each) were diluted using a 2-fold dilution method. The obtained concentrations were investigated for their antibacterial activity using 96 well plates. The first two columns of the well had 100 µl of broth and 100 µl of undiluted phytochemical or antibiotic. The third column up to the eighth column had 100 µl of diluted phytochemical or antibiotic solution in decreasing concentrations and about 100 µl of Staphylococcus aureus suspended in broth. The ninth and tenth column had 200 µl of Staphylococcus aureus cells suspended in broth. The eleventh and twelfth columns had 200 µl of broth. All these components were agitated gently whilst adding each mixture into its respective well. The absorbance of samples in each well was measured using the Elisa Plate reader at 633 nm before incubation. The plate was closed and put in a box with a damp multi-wipe paper. The plate was incubated at 37 ºC overnight. The following day absorbance of each well was measured using the Elisa plate reader and the results were recorded.

RESULTS

Qualitative Screening

Different phytochemicals such as flavonoids, alkaloids, tannins, saponins, and cardiac glycosides were found to be present in Melia azedarach leaf extract as shown in Figure 1.

Figure 1: The tubes show the results for qualitative screening for the phytochemicals. a) shows a brown solution for a positive result for alkaloids by Wagner's reagent, b) a yellow solution for a positive result for alkaloids as well by Meyer's reagent, c) a very pale yellow solution for flavonoids. To a portion of the solution, HCl was added, giving the colourlesss solution in d) proving the presence of flavonoids, e)showing a greenish solution for a positive result for tannins, and f) a white froth forming after shaking for a positive result for saponins. Lastly, g) shows a reddish-brown ring at the conjunction of two mixtures for a positive result for cardiac glycosides.

Quantitative Analysis Results

For each of the phytochemicals that were found present in *Melia azedarach* leaf extract, different procedures were done to effectively quantify them. As for the tannins, absorbance was measured at 650 nm within 10 minutes, and the values obtained were used to produce a graph that was then used to interpolate the mass of the tannins available in the leaves. The Concentration of tannins obtained from the graph was 0.368 g/ml, therefore, using equation :

Tannic acid (mg/100g) = (C * Extract volume * 100)/Aliquot volume * weight of sample
= (0.368g/ml * 50ml * 100)/ 5ml * 2 g
= 1840/10
= 184 mg/100 g

For other phytochemicals, percentage yield for each was calculated as follows:
% phytochemical = (weight of phytochemical/weight of sample) * 100, and results are shown in Table 1

Table 1: Yields of the phytochemicals present in *Melia azedarach* leaves.

Phytochemical	Mass/grams	% content
Flavonoids	0.320	3.2%
Saponins	0.1367	5.47%
Alkaloids	0.300	6%
Tannins **(mg/100g)**	184	

Antibacterial Activity by Disc Diffusion

The tested phytochemicals were found to have antibacterial activity against Staphylococcus aureus as shown in Table 2. Ciprofloxacin was used as the positive control.

Table 2: Susceptibility of *Staphylococcus aureus* to phytochemicals extracted from *Melia azedarach* leaves.

Phytochemical	Inhibition zone/mm
Alkaloids	16 ± 2
Flavonoids	9 ± 2
Tannins	11 ± 2
Saponins	9 ± 2
Ciprofloxacin	13 ± 2

n= 4 for all tested samples

Antibacterial Activity by Broth Dilution

Four different plates were prepared each with a different phytochemical. The phytochemicals were being investigated whether they had antibacterial activity against *Staphylococcus aureus*. The alkaloids had the most potent activity against *S. aureus* with minimum inhibitory concentrations of 50 µg/ml. Thus, alkaloids from *Melia azedarach* leaves may be used as a template for the development of antibacterial agents against *Staphylococcus aureus.*

DISCUSSION

Many studies have been reported for the isolation of active compounds from crude extracts of medicinal plants and it was accepted that the medicinal properties lie in the phytochemicals present in plants. In Zimbabwe, in the African Tradition Religion, the use of herbs is used massively and in the rural areas, the herbs are the source of medical assistance that is known even to the people who do not practice the ATR tradition. In developed countries, the demand for herbal medicines is growing rapidly as it is gaining good acceptance, because of better action and safety profile. The aromatic plants and aromatic chemicals play a vital role directly as well as indirectly in the day-to-day life of man since its appearance on the earth.

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases [12]. The research done in this paper shows that the phytochemicals, that is, alkaloids, saponins, flavonoids, tannins, and cardiac glycosides were present in the leaf extracts of *Melia azedarach.* Flavonoids like baicalin possess an antipyretic effect by suppressing TNFα [12]. Further analysis of the exact type of flavonoids present in the *Melia azedarach* was not done. However, Sultana determined that the flavonoids present in the *Melia azedarach* leaf extracts are Kampherol and Quercetin [13].

Tannins are polyphenolic compounds, which are considered as primary anti-oxidant or free radical scavengers and have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [12]. Alkaloids are well known for their wide pharmacological activities ranging from antibacterial and antifungal activities [7]. Cardiac glycoside, comprises glycosides that exert a cardiotonic action and therefore are used to improve blood circulation and heart function in congestive heart failure.

Medicinal plants play an important role in human life because they possess effective compounds with a high ability to treat diseases. In recent times, many antibiotic-resistant microorganisms have emerged. Therefore, new sources must be required to overcome the resistance of these dangerous microorganisms. Many scientists believe that the solution exists in the plant kingdom. This study proved that the extract from the leaves had antibacterial activity against the bacteria *Staphylococcus aureus.* The results of the research are encouraging as all the tested extracts revealed antibacterial potential, although the inhibitory activity was strain-specific and concentration-dependent. Though the study was on the leaves of the plant, some studies have shown that the seed extracts of *Melia azedarach* are effective in controlling infections caused by both gram-positive and gram-negative strains [14].

CONCLUSION

The phytochemicals isolated from the leaves of *Melia azedarach* have antibacterial activity against *Staphylococcus aureus*. Thus, *Melia azedarach* leaves may be used as a source of compounds for the development of antibacterial agents against *Staphylococcus aureus*.

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