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Phytochemical Analysis and Antifungal Activity of Methanol Extract of *Acacia nilotica* Leaves

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Abstract: ***Acacia nilotica*** **leaves has been used to treat different diseases. In the present study, the leaves of** ***A. nilotica*** **was subjected to phytochemical and antifungal analysis. The extraction was done with methanol using Whatman filter paper No.1 of pore size 11 µm. The phytochemical analysis of the methanol extract of** ***A. nilotica*** **leaves was carried out. The extracts were evaluated for their antifungal activity. The the preliminary qualitative phytochemicals screening of crude methanol extracts of** ***A. nilotica*** **leaves .This showed positive test for alkaloids, glycosides, cardiac glycoside, steroids, saponins, tannins, anthraquinones, flavonoids, terpenoids and phenol. The results for the Mean Standard Deviation composition of Methanol crude extract of** ***A. nilotica*** **presented in Table 2 from the results, Alkaloids and Flavonoids has the highest concentration of (2.090±0.008mg/g), (1.500±0.710mg/g) respectively follow by Saponins with concentration of (0.830±0.006) and then follow by Cardiac Glycoside with concentration of, (0.507±0.008mg/g). The antifungal activities of** ***A. nilotica*** **leaves showed that the extracts possessed antifungal activity at various concentrations, ranging from (90 mg/ml to 30 mg/ml). The best results were obtained on** ***Aspergillus flavus*** **(11.06±0.06 to 6.16±0.88) followed by** ***Aspergillus fumigatus*** **(6.10±0.05 to 2.10±0.05),** ***Candida albicans*** **(5.13±0.03 to 1.06±0.03) and** ***Aspergillus niger*** **(2.13±0.33 to 0.00±0.00). For all the concentration the effect of** ***A. nilotica*** **extract was significantly increases against the fungi species beyond that of Ketoconazole. This study encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators and provide a support to use of plant in traditional medicine.**

Keywords: **Antifungal activity, Methanol extract, Zone of inhibition,** ***Acacia nilotica,*** **Fungi and Phytochemical analysis.**

1. INTRODUCTION

Medicinal plants have been a valuable source of natural active constituents that maintain human health and treatment of many human diseases. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industries.[1], [2].

Acacia nilotica (Babul tree) is the member of the family Mimosaceae; *Acacia nilotica* is multipurpose nitrogen fixing tree legume. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India [3]. Phytochemical analysis of the aerial parts of the plant demonstrated the presence of polyphenolic compounds and flavonoids in the flowers. Tannins, volatile oils, glycosides, coumarins, carbohydrates and organic acids are reported in the fruits [4].

Phytochemical analysis of the aerial parts of the plant demonstrated the presence of polyphenolic compounds and flavonoids in the flowers. Tannins, volatile oils, glycosides, coumarins, carbohydrates and organic acids are reported in the fruits [5]. Babul has been reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyldjenkolic acid, Nacetyldjenkolic acid, sulphoxides pentosan, saponin and tannin. Seeds contain crude protein

18.6%, ether extract 4.4%, fiber 10.1%, nitrogen-free extract 61.2%, ash 5.7%, silica 0.44%, phosphorus 0.29% and calcium 0.90% of DM [6].

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant [7]. There are several reports on the antimicrobial activity of different herbal extracts [8], [9]. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections. Cytotoxic compounds have been isolated from the species of Vismia [10]. According to the World Health Organization (WHO), medicinal plants would be the best source for obtaining variety of drugs [11].

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification.

Mature leaves of *Acacia nilotica* were randomly collected from Nasarawa Area Aliero Local Government, Kebbi State, Nigeria, the sample was later identified and authenticated by Professor Dhramandrah Singh a plant taxonomist at the Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero and was given (Voucher number of 284).

2.2 Preparation of the Leaves Methanol Extract

The plant leaf samples were pulverized to powder using pestle and mortar. 100 g pulverized plant materials were mixed with 1 litre of 95% methanol. The mixture was kept at room temperature for 72 hours and was then filtered with a Whatman filter paper No.1 of pore size 11 µm. The filtrates were evaporated at 45 ^{o}C using water bath. Then the methanol extracted material was dissolved in distilled water and the solution was used for analysis.

2.3 Qualitative Phytochemical Screening

Chemical test were carried out on the methanolic extracts for the qualitative and quantitative determination of phytochemical constituents as describe by [12], [13], [14].

2.4. Quantitative Analysis

2.4.1 Determination of Alkaloids content:

100 cm^3 of 10% acetic acid in ethanol was added to 5 g each of powdered plant sample in a 250 cm^3 beaker and was allowed to stand for 4 hours. The extract was concentrated on the water bath to one-quarter of their original volume followed by addition of 0.1M ammonium hydroxide drop wise until the precipitation was completed. The solution was allowed to settle and the precipitate was collected and washed with 20 cm^3 of 0.1 M ammonium hydroxide and then filtered using filter paper (no.11 $cm^{3)}$, the residue was allowed to dry in oven and weighed. Percentage of alkaloids was calculated mathematically using equation 3.1.

$$\% \text{ Alkaloids} = \frac{\text{weight of the residue}}{\text{weight of the sample}} \times 100 \text{ ----------3.1}$$

2.4.2 Determination of Flavonoids Content

Ten (10) grams of powdered sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was transfer into a crucible and evaporated to dryness over a water bath and weighed. Percentage of flavonoids was calculated mathematically using equation 3.2.

$$\% \text{ Flavonoids} = \frac{\text{weight of dried sample}}{\text{weight of the sample sample}} \times 100 \text{ ----------3.2}$$

2.4.3 Determination of Cardiac Glycosides Content

Cardiac glycosides were determined using the method adopted by Ikeda *et al*.,(1996).5g of the fine powder of plant sample was soaked in 10ml of 70% alcohol for 2hrs. And then filtered. 8ml from the filtrate obtained was mixed with 8 ml of 12 % lead acetate (to precipitate resin, tannin and pigments), this mixture in 100 ml volumetric flask was filled to the mark with distilled water and filtered.50 ml of the filtrate was measured in to another 100 ml volumetric flask, 8 ml of 4.7 % disodium hydrogen phosphate (Na_2HPO_4) solution was added (to precipitate excess lead). The mixture was made

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up to the mark with distilled water. The mixture was filtrated twice with filter paper.10 ml of Buljet's reagent (containing 95ml aqueous picric acid and5ml of 10% aqueous NaOH was added to 10 ml of the filtrates. The blank sample was prepared by addition of 10 ml buljet's reagent in to 10 ml distilled water. The two mixtures were allowed to stand for one hour (time maximum for color development).The intensity of color was read at 495 nm spectrophotometer against the blank The percentage of total glycosides was calculated using the equation 3.3 below.

$$\% \text{ Glycoside} = \frac{\text{Absorbance of the sample}}{17} \times 100 \text{----------} 3.3$$

2.4.4 Determination of Saponins

Five (5g) grams of the sample was placed in a 100ml flask. 60ml of 50% aqueous methanol was added to the content and boiled under reflux for 30 minutes. The resulting solution was filtered while hot using filter paper into a 100ml flask. Two grams (2g) charcoals was subsequently added to the filtrate, further boiled and filtrate while hot. The extract was allowed to cool and equal volume of acetone was added (to complete precipitation of saponins). The precipitate was separated by decantation and then dissolved in 95% ethanol by boiling, filtrate while hot and allowed to cool to room temperature. The filter paper containing the residue was dried in a dessicator and weighed.

$$\% \text{ of Saponin} = \frac{\text{weight saponin residue}}{\text{weight of sample}} \times 100 \text{----------} 3.4$$

2.4 Test Fungal Isolates

Three pathogenic fungi, *Aspergillus fumigatus*, *Aspergillus flavus* , *Aspergillus niger and Candida albicans* were collected from Federal medical centre Birnin Kebbi. The cultures were sub-cultured and maintained on Potato dextrose agar slants and stored in refrigerator at 4 ^{o}C.

2.5 Determination of Antifungal Activity Inoculum Preparation

Fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Potato dextrose and incubated at room temperature for 3 days.

2.6 Determination of antifungal activity by Agar well Diffusion Method

Potato dextrose agar plates were incubated with test organisms by spreading the fungal inoculum on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanol extracts with different concentration (30mg/ml, 60mg/ml and 90mg/ml) were added into the well. Well containing Ketoconazole act as a negative control. The plates were incubated at room temperature for 3 days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm).

3. RESULTS AND DISCUSSION

3.1 Results

The results obtained from the qualitative and quantitative analysis were reported in Table 1 and 2 respectively. The antifungal activity study results were given in Table 3.

Table 1: Quatitative Phytochemical Composition of Methanol Crude Extract of *A. nilotica* Leaves.

Phytoconstituents	Results
Alkaloids	+
Glycosides	+
Cardiac glycoside	+
Steroids	+
Saponins	+
Tannins	+
Anthraquinones	+
Flavonoids	+
Terpenoids	+
Phenol	+

Key: + = Present

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Table 2: Quantitative Phytochemicals Composition of Methanol Crude Extract of *A. nilotica* Leaves.

Phytochemical	Methanol Extract mg/g
Alkaloids	2.090±0.008
Flavonoids	1.500±0.710
Cardiac Glycoside	0.507±0.008
Saponins	0.830±0.006

Table 3: Antifungal Activity of *A. niloticaa* Methanol Leaves Extract

Fungi species	Distilled water	Ketoconazole	Concentration (30mg/ml)	Concentration (60mg/ml)	Concentration (90mg/ml)
A. Niger	0.00 ± 0.00^{a}	7.13 ± 0.03^{a}	0.00 ± 0.00^{a}	1.03 ± 0.33^{a}	2.13 ± 0.33^{a}
A. fumigatus	0.00 ± 0.00^{a}	10.00 ± 0.03^{c}	2.10 ± 0.05^{c}	4.03 ± 0.03^{c}	6.10 ± 0.05^{c}
A. flavus	0.00 ± 0.00^{a}	22.06 ± 0.03^{d}	6.16 ± 0.88^{d}	6.16 ± 0.88^{d}	11.06 ± 0.06^{d}
C. albicans	0.00 ± 0.00^{a}	8.03 ± 0.03^{b}	1.06 ± 0.03^{b}	3.16 ± 0.03^{b}	5.13 ± 0.03^{b}

Values are mean inhibition zones (mm) ± SEM of the three replicate experiments. Mean value having different superscripts letters (abcd) along the rows are significantly different ($P<0.05$) while values with the same superscripts letter in rows, are non significance ($P>0.05$).

3.2 Discussion

Phytochemical screening of methanol extract of *A. nilotica* leaf from Table 1 indicate the presence of certain secondary metabolites such as Alkaloids, Glycosides, Cardiac Glycoside, Steroids, Saponins, Tannins, Anthraquinones, Flavonoids and Terpenoids which may be responsible for the treatment of various disease in human body. This is in agreement with the work of [15], who reported that the crude methanol leave extracts of these plants revealed the presence of glycoside, tannins, phenols saponins and flavonoids. The preliminary phytochemical screening investigation of *A.nilotica* may help in the recognition of bioactive compounds and it may lead to the discovery and development of new drugs. These test will also facilitate separation of pharmacologically active chemical compounds. Therefore *A. nilotica* leafs contain many secondary metabolites which are responsible for various medicinal properties and will be of great importance in phytomedicine like alkaloids which are reported to have many pharmacologigal activities such as Anlgesic, Anti-malarial activity. Tannins may be responsible for the antioxidant activities or free radical scavenging activities and heart diseases prevention as reported by [16]. Steroids are very important compounds due to their relationship with compounds such as sex hormone. The presence of steroids is suggestive of anti-inflammatory activity and blood cholesterol reducing capacity.

The results for the Mean Standard Deviation composition of Methanol crude extract of *A. nilotica* presented in Table 2 from the results, Alkaloids and Flavonoids has the highest concentration of (2.090±0.008mg/g), (1.500±0.710mg/g) respectively follow by Saponins with concentration of (0.830±0.006) and then follow by Cardiac Glycoside with concentration of, (0.507±0.008mg/g).

The presence of the above phytochemicals makes the leaves pharmacologically active; these phyto-constituents may be directly responsible for all medicinal uses as well as the antifungal activity of *A. nilotica.*

The antifungal activities of *A. nilotica* leaves from Table 3 showed that the extracts possessed antifungal activity at various concentrations, ranging from 90 mg/ml to 30 mg/ml. The best results were obtained on *Aspergillus flavus* (11.06±0.06 to 6.16±0.88) followed by *Aspergillus fumigatus* (6.10±0.05 to 2.10±0.05), *Candida albicans* (5.13±0.03 to 1.06±0.03) and *Aspergillus niger* (2.13±0.33 to 0.00±0.00). For all the concentration the effect of *A. nilotica* extract was significantly increases against the fungi species beyond that of Ketoconazole. This result was in agreement with the work of [17] who reported that *Aspergillus flavus* showed hightest activity against *Rizoctoniasolani* which was 41.5±0.57mm followed by *Fusarium solani* 32±0.81mm, *Aspergillus niger* 19.25±0.5 mm, *Pythiummyriotylum* 18.25±0.5mm. [18], also reported that, *Aspergillus flavus* was the dominant species of *Acacia nilotica* have good activity against oral pathogens. As a endophyte *Aspergillus flavus* isolated from Moringa oleifera showed the maximum activity was observe against

Staphylo coccus aureusa and *Bacillus* [19]. As per recent study *Aspergillus flavus* was firstly reported to the highest activity of plant pathogenic fungi i.e *Rhizctoniasolani*, *Fusariumsolani*, *Aspergillus niger* and *Pythiummyriotylum*. Endophytic fungi produce several biologically active metabolites to protect their host life. [18]

Ketoconazole showed higher activity against *A. nilotica* for all the concentration, This was in agreement with the work of [20], Who reported that experimental results showed significant activity against *A. nilotica* in comparison to Ketoconazole (standard drug) in all tested concentrations. [21], Reported that *A. nilotica* showed the maximum antifungal effects against ***Aspergillus niger*** and *Candida albicans*. Additionally, m**ethanolic extract**s prepared from *A. nilotica* leaf demonstrated high inhibitory effects against ***Aspergillus flavus*** growth. [22].

4. CONCLUSION

The findings of this study suggest that the leave extracts of *A. nilotica* showed antifungal activities against *Aspergillus fumigatus, Aspergillus flavus , Aspergillus niger and Candida albicans.* Thus, these plants could serve as potential sources of antifungal agents which might be possibly due to presence of phytochemical constituents in the plant extracts.

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