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Phytochemical Screening and Antidermatophytic Activity of *Caesalpinia pulcherrima* Root Extracts on Selected Dermatophytic Isolates

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Abstract. The analysis of medicinal plants has had a long history, especially with regard to assessing a plant's quality and its efficacy to serve as a source of treatment against skin infection and diseases. Pride of Brabados (*Caesalpinia pulcherrima*) is claimed to have a wide range of therapeutic values due to its application in folkloric medicine. This research work was carried out to determine the antidermatophytic activity of *Caesalpinia pulcherrima* root extract on some selected dermatophytic isolates. Crude aqueous, n-hexane, methanol and ethanol extracts of *Caesalpinia pulcherrima* roots were assayed for their phytochemical constituents and antidermatophytic activity against *Trichophyton rubrum, Trichophyton tonsurans, Microsporum canis, Microsporum audouinii, Epidermophyton floccosum* using agar well diffusion method. Qualitative screening was also carried out on the root of *Caesalpinia pulcherrima* and the results revealed the presence of several phytochemicals such as alkaloids, glycosides, steroids, anthraquinones, terpenoids, tannins, saponins and reducing sugar in which the methanol and aqueous extracts showed the absence of essential oil, and flavanoids, while n-hexane and ethanol showed the absence of terpenoid and essential oil respectively. All extracts derived from *Caesalpinia pulcherrima* roots showed a level antidermatophytic activity against the test isolates at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml). The antidermatophytic effects of *Caesalpinia pulcherrima* roots observed in this study supports the traditional use of the plant in the treatment of infections caused by dermatophytes.

Keywords: antidermatophytic activity, *Caesalpinia pulcherrima*

Introduction

Several medicinal plants have provided mankind a large variety of potent drugs to alleviate or eradicate infections and sufferings from several diseases in spite of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance. The use of plant-based drugs all over the world is increasing (Smet, 1995). There have been records of advances made in the modern (synthetic) medicine but yet there are still a large number of ailments or infection (diseases) for which suitable drugs are yet to be found (Bhat & Jacob, 1995).

This has brought an urgent need to develop safer drugs (both for man and his environment) for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Through recent researches on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine (Bhat & Jacob, 1995).

Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population. Modern medicines and herbal medicines are used in compliment of each other in areas for health care program in several developing countries such as countries in Africa, Asia and some part of Europe (Angell & Kassirer, 1998).

Caeasalpinia pulcherrima also known as Pride of Barbados plant is a perennial large shrub or small tree found throughout the world (Chiang *et al.*, 2003). The continuing popularity

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of *C. pulcherrima* is due to its easy propagation, its adaptability, fast growth, and above all, its brilliant, long-lasting summer blooms. It can be used in variety of ways, including as an ornamental accent plant or hedge and its showy colors attracts birds, hummingbirds and butterflies (Roach, 2003).

The plant *Caesalpinia pulcherrima* is also used in folk medicine; the stem is used as abortifacient and emmenagogue, while decoctions of the roots and bark are used as febrifuge and to treat liver disorders as well as ulcers from the mouth and throats (Oludare *et al.*, 2017). *C. pulcherrima* is also used in the treatment of several illnesses which can be attributed to its possession of several phytochemicals and medicinal properties (Chiang *et al.*, 2003).

Caesalpinia pulcherrima roots is woody in nature, and light brown in color, also the plant root is noted to be an underground (Taproot) that is rigidly fit inside the ground and its connected to the stem and soil of the plant (Clay, 1977).

Methodology

Study Area

The research work was carried out in Anyigba at Kogi State University, which is located in Dekina local government area of Kogi State. It covers a total land area of 29.833km^2, an area rank, 13th of 36 and is located between latitudes 7^{n}30' North and longitude 6^{n}42' East.

Sterilization of Materials

All glass wares used was thoroughly washed with detergent and rinsed with distilled water and was air dried in the hot air oven and then sterilized at 121^{0}c for 15mins. Surfaces of the work bench area were disinfected before carrying out any experimental work in order to avoid contamination and to ensure aseptic working conditions.

Collection of Plant Materials and Identification

The *Caesalpinia pulcherrima* root was collected within Kogi State University Campus, Anyigba in the month of May 2021. Voucher specimens were prepared and the plant materials were identified in the Department of Plant Science and Biotechnology, Kogi State University, Anyigba.

Preparation of Plant Extract

Freshly collected *Caesalpinia pulcherrima* root were cleaned, separated and air dried under normal room temperature. The completely dried root was crushed with the aid of a mortar and pestle, and was then grounded into fine powder.

Sample Extraction

The sample was extracted using two (2) methods:

a) Aqueous extraction

Hundred grams (100g) of powdered root of the plant sample was macerated in 300ml of sterile distilled water for 72hours. The extract was decanted, filtered, concentrated and dessicated using the dessicator to evaporate residual solvents on the water bath as described by Ndip *et al.* (2007).

b) Soxhlet extraction

Hundred gram (100g) of powdered root was packed into the thimble and was place in the Soxhlet extractor and extracted using the solvents N-hexane, ethanol, and methanol respectively. The extract was concentrated using a rotary evaporator for evaporation of residual solvents and weighed after extraction (Williams, 2007; Timothy *et al.*, 2012).

The extract yield and percentage yield for both the methanolic, ethanolic, n-hexane, and aqueous extracts of *Caesalpinia pulcherrima* root were calculated as shown below;

Extract yield (X3) = X1- X2

Where; X1= weight of beaker + crude extract; X2= weight of beaker only.

Percentage yield (%) = (X3÷ weight of powder used) ×100.

Phytochemical Analysis

Phytochemical screening: the following phytochemical tests were carried out:

A) Test for Glycosides
B) Test for Anthraquinones
C) Test for Terpernoids
D) Test for Steroids
E) Test for Alkaloids
F) Test for Saponins
G) Test for Tannins
H) Test for Flavoniods.

Preparation of Culture Media

Saboraud Dextrose Agar (SDA) was used for the microbiological analysis and antidermatophytic activity of crude extract of *Caesalpinia pulcherrima* on the pure culture of the tested dermatophytes. Media was prepared and sterilized according to manufacturer's instruction at ambient laboratory temperature.

Collection of the Test Organism

Laboratory isolates of the test organisms (dermatophytes) was obtained from the stock culture in the laboratory department of Microbiology, Kogi State University.

Preliminary Antidermatophytic Activity of Crude Extracts of *Caesalpinia pulcherrima* Roots

The crude extracts of *Caesalpinia pulcherrima* roots were tested for antidermatophytic activity against the test dermatophytes. Agar incorporation method was used as described by Akinpelu *et al.* (2013). The agar was mixed with the plant extracts and the test organism was inoculated. A control plate without the extract was also set up and was inoculated and was incubated at ambient temperature until growth was apparent and the result was read.

Results

Table 1 shows the extract yield of Aqueous, N-hexane, Ethanol and Methanol extracts of *Caesalpinia pulcherrima* root. The aqueous extracts yielded 3.38g, N-hexane yielded 5.94g, ethanol yielded 5.83g and methanol yielded 6.02g respectively.

Table 2 revealed the presence of various phytochemicals in the Aqueous, N-hexane, Ethanol and Methanol extracts of *Caesalpinia pulcherrima* root.

In the Aqueous extract of *Caesalpinia pulcherrima* root, various phytochemicals such as glycosides, terpenoids steroids alkaloids, tannins, anthraquinone, carbohydrates, phenols and saponins were present while flavonoids and essential oil were absent.

In the N-hexane extract of *Caesalpinia pulcherrima* root, various phytochemicals such as glycosides, steroids, alkaloids, tannins, flavonoids, phenols, carbohydrates, essential oils, anthraquinone and saponins were present while terpenoids was absent.

In the Ethanol extract of *Caesalpinia pulcherrima* root, various phytochemicals such as glycosides, terpenoids, steroids alkaloids, tannins, flavonoids, phenols, Saponins, carbohydrates and anthraquinone were present while essential oil was absent.

In the Methanol extract of *Caesalpinia pulcherrima* root, various phytochemicals such as glycosides, terpenoids, steroids alkaloids, tannins, phenols, saponins, anthraquinone and carbohydrates, were present while essential oil and flavonoids were absent.

Table 3 shows the result of the preliminary antidermatophytic activity of the crude aqueous, n-hexane, ethanol and methanol extracts of *Caesalpinia pulcherrima* root against the test organisms. All the extracts had activity against the tested organisms.

Table 1. The extracts yield for aqueous, n-hexane, ethanol and methanol extracts of *Caesalpinia pulcherrima* root

Extracts	Aqueous	N-hexane	Ethanol	Methanol
Extract yield (g)	3.38	5.94	5.83	6.02

Table 2. Qualitative phytochemical screening of aqueous, n-hexane, ethanol and methanol extracts of *Caesalpinia pulcherrima* root

Phytochemicals	Water	N-hexane	Ethanol	Methanol
Glycosides	+	+	+	+
Terpenoids	+	-	+	+
Steroids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	-	+	+	-
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Carbohydrates	+	+	+	+
Essential oils	-	-	-	-
Anthraquinone	+	+	+	+

Note: + (present); - (absent)

Table 3. Preliminary screening for the antidermatophytic activity of water, n-hexane, ethanol and methanol extracts of *Caesalpinia pulcherrima* root

Organisms	**Extracts**		
	Aqueous N-hexane	Ethanol	Methanol
Epidermophyton floccosum	- -	-	-
Microsporum canis	- -	-	-
Microsporum audouinni	- -	-	-
Trichophyton	- -	-	-
Rubrum			
Trichophyton	- -	-	-
Tonsurans			

Note: + = Growth; - = No growth

Table 4 shows the antidermatophytic susceptibility of *Caesalpinia pulcherrima* root aqueous extract at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Table 5 shows the antidermatophytic susceptibility of *Caesalpinia pulcherrima* root N-hexane extract at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Table 7 shows the antidermatophytic susceptibility of *Caesalpinia pulcherrima* root methanol extract at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Table 4. Antidermatophytic susceptibility of aqueous extracts of *Caesalpinia pulcherrima* root at different concentrations

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Positive Control (Ket) 25mg/ml	Negative control (water)
Epidermophyton floccosum	16	14	12	10	8	0.0
Microsporum canis	18	16	14	12	10	0.0
Microsporum audouinii	17	14	12	10	8	0.0
Trichophyton rubrum	17	15	13	11	9	0.0
Trichophyton tonsurans	16	14	12	10	8	0.0

Note: Ket – Ketoconazole (25mg/ml)

Table 5. Antidermatophytic susceptibility of N-hexane extracts of *Caesalpinia pulcherrima* root at different concentrations

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Positive Control (Ket) 25mg/ml	Negative control (water)
Epidermophyton floccosum	15	13	11	9	8	0.0
Microsporum canis	16	14	12	10	10	0.0
Microsporum audouinii	16	13	11	9	8	0.0
Trichophyton rubrum	15	13	11	9	9	0.0
Trichophyton tonsurans	14	12	10	8	8	0.0

Note: Ket – Ketoconazole (25mg/ml)

Table 6. Antidermatophytic susceptibility of ethanol extract of *Caesalpinia pulcherrima* root at different concentrations

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Positive Control (Ket) 25mg/ml	Negative control (water)
Epidermophyton floccosum	18	16	14	12	8	0.0
Microsporum canis	20	18	16	14	10	0.0
Microsporum audouinii	20	17	15	13	8	0.0
Trichophyton rubrum	19	17	14	12	9	0.0
Trichophyton tonsurans	20	18	16	13	8	0.0

Note: Ket – Ketoconazole (25mg/ml)

Table 7. Antidermatophytic susceptibility of methanol extracts of *Caesalpinia pulcherrima* root at different concentrations

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Positive Control (Ket) 25mg/ml	Negative control (water)
Epidermophyton floccosum	20	18	16	14	8	0.0
Microsporum canis	22	20	18	16	10	0.0
Microsporum audouinii	21	19	17	15	8	0.0
Trichophyton rubrum	22	20	18	16	9	0.0
Trichophyton tonsurans	21	19	17	15	8	0.0

Note: Ket – Ketoconazole (25mg/ml)

Discussion

Medicinal plants contain a wide variety of secondary metabolites which has subjected several plants of medicinal importance to screening in a bid to discover new drugs that can be effective in the treatment of various diseases including dermatophytosis (Alim *et al.,* 2009). Medicinal plants have been the basis of treatment of various diseases in African traditional medicine as well as other forms of treatment from diverse cultures of the world (Theophine *et al.,* 2014).

It was observed in this study that the aqueous, n-hexane, ethanolic, and methanolic extracts of *Caesalpinia pulcherrima* roots contain various phytochemicals known to exhibit a variety of biological activities such as antidermatophytes (Odunbaku & Lusanya, 2011) and these phytocompounds were reported to have different modes of actions.

The qualitative results of the phytochemical analysis of water, n-hexane, ethanolic and methanolic extract of *Caesalpinia pulcherrima* roots revealed the presence of Glycosides, terpenoids, flavonoids, tannins, steroids, Alkaloids, and phenols (Table 2) showing the absence of essential oils. From this result, it can be shown that the presence of these active compounds was responsible for the antidermatophytic effects exhibited by *Caesalpinia pulcherrima ro*ots. This result corresponds with the epidemiological investigation by Oyerinde *et al.* (2013) who reported that the presence of these phytochemicals may be responsible for the bioactive properties of the extracts. Phytochemicals generally exert their antifungal activity through different mechanisms from that of synthetic drugs (Schuemie *et al.,* 2018).

Also in this study, the aqueous, ethanol and methanol extracts of *Caesalpinia pulcherrima* roots were evaluated for their antidermatophytic activity against the selected dermatophytes, this results corresponds to that of (Akash *et al.,* 2020). This research for the phytochemicals derived from the plant roots has effects on medicine in controlling the growth of microorganisms (Ahmed *et al.,* 2012). The growth inhibition of the test organisms in this study showed strong dependent effects on the various extracts concentration. The antidermatophytic susceptibility of the plant is in accordance with the decrease in concentration and further increase following an increase in concentrations. The extracts of *Caesalpinia pulcherrima* roots showed higher growth inhibition at 25mg/ml (18mm) compared to Ketoconazole (10mm).

Several reports of the antimicrobial activities against human pathogens have been widely carried out on *Caesalpinia pulcherrima* (Hofilena *et al.,* 2000). The methanolic and aqueous extracts of Caesalpinia pulcherrima were observed to have reasonable antidermatophytic effect on the tested dermatophytic isolates (i.e. *Epidermatophyton floccusum, Trichophyton tonsurans, Trichophyton rubrum, Microsporum canis, and Microsporum audounii*) as shown in Table 3. This agrees with several reports in which similar observations were made (Fawehinmi *et al.*, 2013).

Thus, the antidermatophytic effects exhibited by the methanolic and aqueous extract of *Caesalpinia pulcherrima* roots as seen in this study may also be due to their phytochemical content which caused inhibitory effects on the mycelia growth of the fungi due to the leaks in the cell wall or perhaps some alterations in membrane permeability resulting in loss of cytoplasmic constituents (Phongpaichit *et al.,* 2004). In addition, this finding confirms the traditional therapeutic claims for *Caesalpinia pulcherrima* use in treatment of ringworm and skin diseases.

Conclusion

The results of this study revealed that secondary metabolites are rich in *Caesalpinia pulcherrima* root and they can act as antidermatophytic compounds against the growth of species of all three genera of dermatophytes. Further, these secondary metabolites can be used in treatments as active ingredients for many herbal medicines. Thus, the efficacy of secondary

metabolites which exhibit antidermatophytic activity against dermatophytes in plants such as *Caesalpinia pulcherrima* can be further studied under clinical condition.

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