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PHYTOCHEMICAL CONSTITUENTS AND ANTIFUNGAL ACTIVITY OF ACALYPHA WILKESIANA LEAVES ON CANDIDA ALBICANS

***Asmau Nna Sulaiman, Adole Emmanuel James**

Department of Microbiology, Ahmadu Bello University Zaria

*Corresponding authors' email: sulaimanasmau@yahoo.co.uk

ABSTRACT

Acalypha wilkesiana, commonly called Jacob's coat, is native to South Pacific Islands and belongs to the family Euphorbiaceae. This study was conducted to evaluate the phytochemical constituent and the antifungal effect of methanolic and aqueous extracts of *Acalypha wilkesiana* leaves on *Candida albicans.* The aqueous and methanolic extracts of *Acalypha wilkesiana* were investigated for their antifungal activity against *Candida albicans* using agar well diffusion method. A total of twenty (20) isolates were collected from Ahmadu Bello University Medical Centre, Samara, Zaria, of which all were confirmed using microscopy (wet mount), culture and Germ tube test to be *Candida albicans.* The plant extract was prepared using maceration method. The preliminary phytochemical screening of the extract showed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids. The Minimum Inhibitory Concentration for both the aqueous and methanolic extracts were at 100 mg/mL and 75 mg/mL. The Minimum Fungicidal Concentration for both extracts were at 100 mg/mL. *Acalypha wilkesiana* studied here can be seen as a potential source of useful drugs. The result of this study therefore justifies the use of plant as the herbal medicine for the treatment of fungal infection caused by *Candida albicans*.

Keywords: *Alcalypha wilkesiana*, *Candida albicans*, Candidiasis, fungal infection, vagina

INTRODUCTION

Candidiasis is caused by yeast of the genus *Candida* which are part of the normal flora in healthy host confined to the skin and mucosal cavity of the oral cavity, gastrointestinal, urogenital tracts, and vagina (CDC, 2020). It is the most prevalent systemic mycosis mostly caused by *Candida albicans, Candida parapsilosis, Candida glabrata, Candida tropicalis, Candida guilliermondii,* and *Candida dubliniensis* which result to systemic and cutaneous infection. *Candida albicans* and *Candida glabrata* species colonize the mucosal surfaces of all humans immediately after birth where they persist as opportunistic fungi causing infection. Candidiasis often involves the skin or mucus membranes owing to the fact that *Candida* is a typical aerobe and grows very well on such surface. Cutaneous involvement usually occurs when the skin becomes moist or damaged (Wächtler *et al.,* 2012).

In many developing countries, plants are used in the treatment of infections such as Candidiasis. This is due to the presence of biologically active compounds in the plants, their efficacy, cost effectiveness and lesser side effects compared to synthetic forms (Amadioha, 2002).

Acalypha wilkesiana is commonly known as copperleaf and Jacob's coat and is of the family Euphorbiaceae. Other names include Beef steak plant, fire bush, Fijian fire plant. It is a fast- growing evergreen shrub that produce a continuous splash of colours on the land ranging from bronze red to muted red, green, purple, yellow, orange, pink or white, depending on the cultivar. It is widely cultivated and separate male and female flowers appear on same plant and produce fruit all year round. (Gilman, 2000). The continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms is crucial for the advancement of chemotherapy. The investigation of some indigenous plants for their antimicrobial properties may yield useful results (El astal *et al.,* 2005). Many studies have shown that some plants have substances such as peptides, unsaturated long chain aldehydes, alkaloids, essential oils, phenolics, as well as different ethanol, chloroform, methanol and butanol soluble compounds. These plants have emerged as plants with compounds possessing significant therapeutic potential against human pathogens, including bacteria, fungi or virus (El astal *et al.,* 2005).The aim of this study was to evaluate the phytochemical constituent and the antifungal effect of aqueous and methanolic extracts of *Alcalypha wilkesiana* leaves on *Candida albicans*

MATERIALS AND METHODS

Study area

This study was carried out at Ahmadu Bello University, Zaria, Longitude 7° 38' N and Latitude 11° 11'E at an elevation of 722m above sea level with a population of 77, 662 and a hot climate (Latlong, 2021).

Ethical clearance and approval

Ethical clearance was obtained from the University Health Service Centre, Ahmadu Bello University Medical Centre, Zaria- Nigeria.

Sample size

Non probability sampling based was done where a total of 20 isolates of *Candida albicans* were collected from the University Medical Center, Ahmadu Bello University, Zaria-Nigeria.

Confirmation of test organism

Cultural characterization of isolates

The presumptive isolates suspected to be *Candida albicans* collected from Ahmadu Bello University Medical Centre, were sub cultured in Sabouraud Dextrose Agar with the addition of 0.02g of 50mg chloramphenicol at 37°C for 24-48 hours. *Candida albicans* appeared creamy with pasty colonies, opaque, slightly domed, smooth and white colonies on Sabouraud Dextrose Agar (Willey *et al.,* 2008).

Direct microscopy

An isolate suspected to be *Candida albicans* was mixed with a drop of normal saline to make a suspension. A drop of the

suspension was placed on a clean, grease free glass slide, and covered with a coverslip then observed under the light microscope for yeast cell, hyphae with budding cells (Willey *et al*., 2008; Frobenius and Bogdan, 2015).

Germ tube test

About 0.5mL of human serum was added into a test tube, and a colony of the yeast was emulsified in it and it was then incubated at 37°C for 3 hours. A drop was placed on a clean, grease free glass slide and covered with a coverslip. It was observed using 10X and 40X objective (Murray *et al.*, 2007). A yeast cell that had a short, slender hyphal (filamentous) extension laterally that had no constriction at the point of origin with tube-like structures (germ tube) indicated *Candida albicans* (Priya and Pandian, 2020).

Plant material

Collection and identification of plant material

Fresh leaves of *Acalypha wilkesiana* were collected from the Botanical Garden of Biological Science Department, Ahmadu Bello University, Zaria- Nigeria and taken to the Herbarium unit of Biological Science Department, Faculty of Life Sciences for identification and authentication with voucher number ABU03270.

Preparation of plant materials

The leaves were washed thoroughly three time under running tap water, then rinsed with sterile distilled water. It was air dried at room temperature for about 3 weeks, after which it was pulverized into fine powdered form using mortar and pestle and was stored at room temperature for the purpose of extraction.

Extraction of plant material

Methanolic extraction

Methanolic extraction of the leaves of *Acalypha wilkesiana* was carried out at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria- Nigeria using maceration method. About 50 grams of the powdered plant material was soaked in 500 mL of methanol. The mixture was shaken for about 6 hours using a mechanical shaker at room temperature, at constant stirring rate of 200 rpm for an even mixture. It was then left for 24 hours after which it was filtered through a filter paper (Raaman, 2006; Azwanida, 2015).

A*queous extraction*

Aqueous extraction of plant material was carried out using same procedure for the methanolic extract, in this case using sterile distilled water (Raaman, 2006 and Azwanida, 2015).

Phytochemical screening

The extracts were screened to check for the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroids, phenolic compounds, carbohydrates, terpenoids and anthraquinone using the method described by Trease and Evans (1989); Sofowora, 1993. These tests were carried out at the Department of Pharmacognosy and Drug development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria- Nigeria.

Test for Anthraquinones

Bontrager's test

About 10 mL of benzene was added to 6g of the powdered sample in a conical flask and soaked for 10 minutes and then filtered. 10 mL of 10% ammonia solution was added to the filtrate and shaken vigorously for about 30 seconds. Absence of violet, pink, to red colour indicated the absence of anthraquinones in the ammonia phase (Trease and Evans, 1989).

Test for Tannins

Bromine Water test

About 10 mL of bromine water was added to the 0.5 g of extract. Decolouration of bromine water showed the presence of tannins (Trease and Evans, 1989).

Test for Saponins

Frothing test

About 5.0 mL of sterile distilled water was mixed with 0.5 g of plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins (Trease and Evans, 1989).

Tests for Flavonoids

Alkaline Reagent Test.

About 2 mL of 2% NaOH mixture was mixed with 0.5g of plant extract; concentrated yellow colour was produced, which became colourless on addition of 2 drops of diluted acid to mixture. This result showed the presence of flavonoids (Trease and Evans, 1989).

Tests for Cardiac Glycosides

Keller-Kiliani Test.

About 4.0 mL of glacial acetic acid solution and 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 mL plant crude extract and exactly 1 mL of concentrated H_2SO_4. A brown ring formed in between the layers which showed the presence of cardiac steroidal glycosides (Sofowora, 1993).

Test for Terpenoids

Liebermann's Test.

About 2.0 mL of acetic acid and chloroform each, was added to 0.5g of plant extract and then heated. The mixture was then cooled and concentrated H_2SO_4 was added. Appearance of green colour showed the presence of aglycone, steroidal part of glycosides (Trease and Evans, 1989).

Test for Steroids

Salkowski's Test.

About 2 mL of concentrated H_2SO_4 was added to 1 g of plant extract. A reddish brown colour formed which indicated the presence of steroidal aglycone part of the glycoside (Trease and Evans, 1989).

Test for Carbohydrates

Molisch test

Few drops of Molisch's reagent was added to the solution of the extract followed by addition of concentrated H_2SO_4 from the side to form a lower layer. Violet ring at interface indicate the presence of carbohydrates (Trease and Evans, 1989).

Test for Phenols

Lead acetate test

Few drops of lead acetate solution were added to the solution of the extract. A yellow- coloured precipitation indicates phenols (Trease and Evans, 1989).

Test for Alkaloids

Mayer's test

Few drops of Mayer's, reagent was added to the solution of the extract. The presence of cream precipitate indicates the presence of alkaloids (Trease and Evans, 1989).

Preparation of test concentration

Serial dilution was made to obtain the different concentration of the extract, 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5mg/mL by dissolving 5g of the extract in 10 mL of distilled water to obtain a 500mg/mL concentration of the extract. 2 mL of 500 mg/mL concentration was diluted in an equal volume of distilled water to obtain a 250 mg/mL concentration. The serial dilution procedure was continued to obtain lower concentration of the extract as 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL. Sterile distilled water was used as negative control while fluconazole (200mg) was used as positive control (Amadioha, 2002).

Standardization of inoculum

About 0.5 Mc Farland standard was prepared by adding 0.6mL of 1% Barium chloride solution to 99.4mL of 1% sulphuric acid, it was then mixed thoroughly. A small volume of turbid solution of the mixture was transferred to another tube and was capped. It was stored at room temperature. A colony obtained from pure culture of the test organisms was transferred to a sterile bottle containing normal saline and shaken well in order to obtain a homogeneous mixture. The mixture was compared with 0.5 McFarland turbidity standard to obtain a density equivalent to 1.5×10^8 CFU/mL (Cockerill *et al.,* 2012).

Antifungal activity of the extract against *Candida albicans*

The antifungal activity of the plant extract was tested on the *Candida albicans* on Sabouraud Dextrose Agar using agar well diffusion method as described by Bauer *et al.,* 1966. About 0.1mL of the standard inoculum was pipetted and dispensed on the surface of the Sabouraud Dextrose Agar plates. A sterile bent rod was used to spread the dispensed standard inoculum evenly on the surface of the agar plate. The agar was then incubated at room temperature for 10 minutes after which a sterile cork borer of 5mm was used to make 7 wells for each concentration of the extracts on different Sabouraud Dextrose Agar plates containing each of the suspected isolates of *Candida albicans*, the wells were labelled appropriately. Micro titre pipette was used to measure 0.1mL of each extract concentration: 500mg/mL, 250mg/mL, 125mg/mL and 62.5mg/mL dispensed into four wells. Also, 0.1mL of each of the negative and positive control (sterile distilled water and fluconazole (200mg)) respectively was dispensed into the fifth and sixth well respectively. The plates were kept for about 30 minutes to allow pre-diffusion of the extract into the media. The plates were incubated at 37^{o}C for 24-48 hours. Appearance of zone of inhibition around the wells were measured to the nearest millimeter using a ruler.

Determination of minimum inhibitory concentration (MIC)

In determining the antifungal activity of *Acalypha wilkesiana*, the minimum fungal growth inhibition was accessed using both the methanolic extract and the aqueous extract. 2 mL of Sabouraud Dextrose Broth was pipetted into test tubes for both extract concentrations of 25 mg/mL, 50mg/mL, 75mg/mL and 100mg/mL respectively. 25mg/mL, 50mg/mL, 75mg/mL and 100mg/mL of the extract and fractions were added to different test tubes containing the Sabouraud Dextrose Broth. This was prepared for each organism and done in duplicate. A colony of 24 hour cultured organism was inoculated into test tube containing 1 mL of normal saline to give a turbidity of 0.5 McFarland standard and was later dispensed into the test tube containing the suspension of Sabouraud Dextrose Broth, for the both extract and the various fractions of the extracts. This was done for all the organisms at the different concentrations. All test tubes were properly corked and incubated at 37^{0}C for 24-48 hours. After which they were observed for absence or presence of visible growth. The lowest concentration showing no visible growth was recorded as the Minimum Inhibitory Concentration (MIC) (Amadioha, 2002).

Determination of minimum fungicidal concentration (MFC)

The tubes which showed no visible growth from the Minimum Inhibitory Concentration test was sub-cultured onto sterile Sabouraud Dextrose Agar plates, and incubated at 37^{0}C for 24-48 hours. The lowest concentration of the extract that yields no growth were recorded as the Minimum Fungicidal Concentration (Amadioha, 2002).

RESULTS

The cultural characteristics, microscopy and germ tube test for *Candida albicans* were found to be creamy, opaque, slightly doomed, smooth and white colonies on Sabouraud Dextrose Agar. Appearing as yeast cells, hyphae with budding cells in wet mount while in Germ tube appeared as a short hyphal extension arising from yeast cell with no constriction at point of origin. This is indicated in Table 1.

Table 1: Cultural characteristics, microscopy and germ tube test of the isolates

Cultural characteristics	Wet mount	Germ tube	Inference
Creamy, opaque, slightly doomed, smooth and white on Sabouraud Dextrose Agar.	Yeast cell, hyphae with budding cells.	A short hyphal extension arising from a yeast cell, with no constriction at the point of origin	*Candida albicans*

The aqueous and methanolic extracts of *Acalypha wilkesiana* were found to contain saponins, alkaloids, cardiac glycosides, tannins, steroids, carbohydrate, flavonoids, phenolic compounds and terpenoids. Anthraquinone was absent in both extracts. This is indicated in Table 2.

Table 2: Phytochemical constituents of *Acalypha wilkesiana* leaves extracts

Phytochemical constituents	Methanol	Aqueous
Alkaloids	+	+
Cardiac Glycosides	+	+
Saponins	+	+
Phenolic compounds	+	+
Tannins	+	+
Steroids	+	+

Carbohydrates	+	+
Flavonoids	+	+
Terpenoids	+	+
Anthraquinones	-	-

KEY: + = Presence
- = Absence

The antifungal activity of aqueous and methanolic extracts of *Acalypha wilkesiana* on *Candida albicans* showing the mean zone of inhibition (mean ± standard deviation) of *Candida albicans* in aqueous and methanolic extract of *Acalypha wilkesiana* was highest at 19.6 ±2.66 mm at a concentration of 500 mg/mL and 18.4 ± 1.79 mm at a concentration of 500 mg/mL respectively. This was indicated in Table 3.

Table 3: Antifungal activity (mean ± standard deviation) of aqueous and methanolic extracts of Acalypha wilkesiana leaves on Candida albicans

Concentration (mg/mL)	**Zone of inhibition (mm)**	
	Aqueous extract	**Methanolic extract**
500	19.6 ±2.66	18.4 ± 1.79
250	15.05 ± 1.99	14.8 ± 2.12
125	10.05 ± 1.73	11.1 ± 2.27
62.5	5.00 ± 0.00	5.00 ± 0.00
31.25	5.00 ± 0.00	5.00 ± 0.00
Sterile distilled water (Negative control)	5.00 ± 0.00	5.00 ± 0.00
Fluconazole (200mg) (Positive control)	24.95 ± 1.70	26.45 ± 2.67

The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of aqueous and methanolic extract of *Acalypha wilkesiana* against *Candida albicans* was indicated in Table 4. It showed MIC at 75 mg/mL and 100mg/mL for both the aqueous and methanolic extracts and shows an MFC of 100 mg/mL for both extracts.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of aqueous and methanolic extracts of *Acalypha wilkesiana* against *Candida albicans*

Isolate code	**Concentration (mg/mL)**			
	Aqueous extract		**Methanolic extract**	
	MIC	**MFC**	**MIC**	**MFC**
P1	100	-	100	-
P2	75	100	100	-
P3	100	-	100	-
P4	100	-	75	100
P5	75	100	75	100
P6	100	-	100	-
P7	75	100	75	100
P8	75	100	75	100
P9	75	100	75	100
P10	75	100	100	-
P11	100	-	100	-
P12	100	-	100	-
P13	75	100	100	-
P14	75	100	75	100
P15	100	-	100	-
P16	100	-	75	100
P17	100	-	100	-
P18	75	100	100	-
P19	100	-	75	100
P20	75	100	100	-

DISCUSSION

The cultural, Germ tube test and microscopic finding confirmed the test isolates to be *Candida albicans* which appeared on Sabouraud Dextrose Agar (SDA) as a creamy, slightly doomed, smooth white colonies, this supports the description by Willey *et al*., (2008). Priya and Pandian, 2020; Frobenius and Bogdan, (2015) reported that *Candida albicans* have short hyphal extensions arising from the yeast cell with no constriction at the point of origin which is similar to the findings in this work.

The phytochemical screening revealed the presence of saponins, alkaloids, cardiac glycosides, tannins, flavonoids,

steroids, carbohydrates, terpenoids and phenolic compounds while anthraquinone was found to be absent in both the aqueous and methanolic extract. This finding is similar to that of Oladunmoye, (2006) and Haruna *et al.,* (2013). The activity of the extract may be due to these bioactive components such as phenols known to be anti-mutagenic, antimicrobial, anti-carcinogenic and anti-inflammatory and flavonoids known as nature's biological response modifiers (Azwanida, 2015) amongst others.

Also, the antifungal activity of aqueous and methanolic extracts of *Acalypha wilkesiana* on *Candida albicans* had a mean zone of inhibition (mean ± standard deviation) which was highest at 19.6 ± 2.66 mm for aqueous extract and 18.4 ± 1.79 mm for methanolic extract both at 500 mg/mL. The reason for the high activity of the aqueous extract compared to the methanolic extract probably may be due to its high polarity and the ability of solvents to dissolve in it. Although, fluconazole (200mg) which was used as positive control had more activity. This finding is in line with that of Kingsley and Marshall, (2014) and Anokwuru *et al*., (2015).

The Minimum Inhibitory Concentration for both aqueous and methanolic extracts were at 75 mg/mL and 100 mg/mL, while the Minimum Fungicidal Concentration (MFC) of the aqueous and methanolic extracts of *Acalypha wilkesiana* on *Candida albicans* was 100 mg/mL. Ten (10) of the isolates had MFC at 100 mg/mL and 10 isolates also had no fungicidal activity for the aqueous extract. The methanolic extract showed Minimum Fungicidal Concentration (MFC) against *Candida albicans* at 100 mg/mL for 8 isolates while 12 isolates had no fungicidal activity. This may be due to the fact that the concentrations of the extracts were not high enough to completely inhibit the growth of the fungal isolates and cause a fungicidal effect.

Many studies have established the usefulness of medicinal plants as a great source for the isolation of active principles for drug formulation. Several species of the genus *Acalypha* have been studied and it has been demonstrated that they have antifungal, wound healing, antioxidant, post-coital neutralization of venom, antibacterial, antifertility, and anti trypanosomal activities (Murray *et al*., 2007). The results of this study support the antifungal activities of *Acalypha wilkesiana* as an antifungal agent since it inhibited the growth of *Candida albicans.* The finding of this study revealed the antifungal activity of *Acalypha wilkesiana* against *Candida albicans* shown by the zones of inhibition which support the work of Haruna *et al.,* (2013); Kingsley and Marshall, (2014) who also reported the medicinal potential of *Acalypha wilkesiana*. The fact that the aqueous and methanolic extract of *Acalypha wilkesiana* showed activity against the test organisms is a major discovery in appreciating the medicinal potential of the plant. Ezekiel *et al*. (2009) also reported the antifungal activity of the plant extract against *Candida albicans* which is in support of the findings of this research.

CONCLUSION

The phytochemical constituents of both aqueous and methanolic extract of *Acalypha wilkesiana* obtained in this study include alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terepnoids while anthraquinones was absent. The antifungal activity of the plant extracts was highest highest for both aqueous and methanolic extract. The MIC and MFC were determined for both extracts although some had no fungicidal activity. The study highlighted the therapeutic potential of both aqueous and methanolic extract of *Acalypha wilkesiana* against *Candida albicans* with the aqueous extract showing more activity. The clear zones of inhibition against the test isolates indicated that *Alcalypha wilkesiana* has great potentials as antifungal agent against *Candida albicans* and hence can be used after fractionating and purification of the key compound responsible for its activity . The various phytochemical compounds detected are known to have beneficial use in industries and medical sciences, and also exhibit physiological activity. The plant (*Acalypha wilkesiana*) studied here can be seen as a potential source of useful drugs.

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