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Phytochemical Analysis and Antifungal Activity of Leaf Extracts of *Senna alata* (L) Roxb (Leguminosae) Against *Candida albicans*

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ABSTRACT

Candida albicans causes human opportunistic infections. *Senna alata* (Leguminosae) leaves have been utilized in traditional medical practice to treat infectious diseases. The current research aimed to determine the bioactive compound in *Senna alata* leaves against *Candida albicans*. Qualitative and quantitative phytochemical investigations were done on the crude aqueous leaf extract of *Senna alata* to test the occurrence of phytochemical constituents in the extract. The leaves of *Senna alata* were extracted with various solvents (petroleum ether, methanol and water) with the aid of the soxhlet apparatus. The aqueous extract was partitioned with ethyl-acetate, diethyl ether and water to obtain the corresponding fractions. The extracts and fractions were investigated for activities against *Candida albicans.* Column and thin-layer chromatography (TLC) were employed to analyze the diethyl ether fraction and its sub-fractions, respectively. The diethyl ether TLC spot was subjected to infrared (IR) and gas chromatography-mass spectroscopic (GC-MS) investigations. The qualitative phytochemical investigation of the aqueous *Senna alata* leaf extract displayed alkaloids, phenolic compounds, tannins, carbohydrates, reducing sugar, polyuronides, coumarins, saponins, steroids, triterpenoids, flavonoids, resins, cyanophores, cardiac glycosides and anthraquinones. However, carotenoids were absent. The quantitative phytochemical investigation of the aqueous *Senna alata* leaf extract revealed that 10 g of the powdered leaves contained 1.27 g±0.00 (12.7%±0.01) of flavonoids, 0.64 g±0.01 (12.8%±0.01) of alkaloids, 0.004 g±0.00 (0.9%±0.00) of cyanogenic glycosides, 0.30 g±0.02 (1.48%±0.02) saponins and 0.28 g±0.01 (9.6%±0.01) of tannins. The aqueous extract and diethyl ether fraction exhibited the highest effect against the *Candida albicans*. Chromatographic column analysis of the diethyl ether fraction yielded 13 eluates; whereas, the TLC analysis of the sub-fraction 13 revealed three spots. The spectral result obtained for peak 3 of the active TLC spot corresponds with the data from the IR study. The compound related to 9-octadecenoic acid methyl ester was identified. *Senna alata* leaves could possess bioactive compounds with promising antifungal activities against *Candida albicans*.

Keywords: antifungal activity, bioactive compound, fractionation, 9-octadecenoic acid methyl ester, medicinal plant, *Candida albicans, Senna alata*

INTRODUCTION

Disease-causing organisms have been shown to developed resistance to antimicrobial agents [1]. Several researchers reported that wide applications of antimicrobial compounds have led to the resistance by the pathogens, which was further complicated by the development of

cross-resistance [2]. The incidence of yeast infections has been increasing globally [3]. Treatment of fungal infections such as candidiasis has been facing challenges by the limited antifungal agents such as polyenes, azoles, allylamines and echinocandins [4]. *Candida albicans* has been reported to be the leading fungal infections, resulting in life-threatening infections such as oral thrush, vaginitis, actinomycosis, nocardiosis and invasive candidiasis, particularly in immunocompromised patients admitted with serious underlying health conditions, including acquired immune deficiency syndrome (AIDS), patients on cancer chemotherapy, and organ or bone marrow transplantation [5,6].

The use of herbal products has obtained popularity in traditional practice and serves as a main source of drugs from natural origin used by many people, particularly in developing communities [7]. About 30% of the currently utilized medications consist of therapeutic agents sourced from plants [8,9]. It was reported that close to 80% of the people at the global level depends on plant-derived medications as a basis of their basic health care requirement [10]. Therefore, there is a need for pharmacological evaluation of herbal products to motivate the discovery of novel and effective medicinal products for treating various diseases [11]. Looking at the limited therapeutic options for treating fungal infections and antifungal resistance developed by Candida species such as *Candida albicans*, there is an urgent requirement for a new treatment strategy against fungal infections [12]. Several medicinal plants and their products were effective against fungal infections [13,14]. Therefore, evaluating the plant-based bioactive agents against fungal infections could be necessary to isolate and discover novel antifungal agents [12].

The plant *Senna alata* (Linn) Roxb (synonym: *Cassia alata*) belongs to the Leguminosae family. This plant is available in Asia, Brazil, Australia, and Africa [15]. It is an erect annual herb that grows up to 2-5 meters high in a tropical environment with large and slippery leaves which are bilateral [16]. The fruit of the plant is pod-shaped, while the seeds are tiny and square [17]. The plant is called craw-craw, Acapulco, candle bush, or ringworm bush plant (English) [15]. In Nigerian languages, it is called in Yoruba as *Asunwonoyinbo*, Igbo as Nelkhi, and Hausa as *FilaskonMakka* or *Hantsi* [16].

The plant, *Senna alata,* has been used to manage liver diseases, gastrointestinal problems (diarrhoea, constipation, gastroenteritis), dermatological disorders (dermatitis and eczema) jaundice, [17], and bacterial infections [18]. The stems, leaves, and roots of the plant are used to manage wounds, respiratory tract and dermatological infections in Nigeria [17]. The leaves decoction has been utilized to treat epilepsy and skin infections in Western-Nigeria

[19]. Besides, the leaves, stem and bark of *Senna alata* are used against gastroenteritis, dermatological infections, parasitosis and syphilis [15,20]. The diseases caused by pathogenic microorganisms have been posing serious health challenges worldwide, particularly in economically constrained nations. Plant-sourced bioactive molecules play an essential role in discovering active agents for use against infective organisms [26]. Hence, there is a strong need for more investigations to discover phytochemical compounds with potential bioactivity against infective agents [27,28]**.** The present work determined the phytoconstituents, and bioactive compounds from *Senna alata* leaves that could possess antifungal activities against drug resistant *Candida albicans* as a basis to develop novel anti-infective agents with promising activity against fungal infections. On the basis of the documented traditional indication of the *Senna alata*, it has been shown that the plant could possess potential medicinal efficacy against infectious agents.

Therefore, the current research was conducted to determine the possible effectiveness of the leaf extracts from *Senna alata* against drug-resistant *Candida albicans* and determine the active phytocompounds that could have antifungal activity.

MATERIALS AND METHODS

Collection and botanical authentication of the plant

The *Senna alata* leaves were sourced from Jaji, along Kaduna-Zaria road, Kaduna State, Nigeria, and authenticated at the Herbarium facility of the Department of Biological Sciences, Ahmadu Bello University (ABU), Kaduna State, Nigeria. The specimen was compared with an already kept voucher (Voucher number: 2421).

Test organisms

The test organism used for the experiment was *Candida albicans* (clinical isolate) obtained from the Department of Microbiology, ABU, Zaria, Kaduna State, Nigeria.

Extraction and fractionation

The experimental method employed by Kupchan *et al*.[21] was used for the extraction and fractionation. The *Senna alata* leaves were shade-dried with frequent weighing until a constant weight was achieved. The dried leaves were size-reduced using mortar and pestle. One kilogramme (1 kg) of the dried and fine powdered *Senna alata* leaves was extracted with 2.5 litres of petroleum ether (60-80 grades) with the aid of the soxhlet apparatus. The marc of the petroleum ether extract was subsequently extracted with 2.5 litres absolute methanol with Soxhlet apparatus. Then the marc of the methanolic extraction was dried and percolated with

1 litre of deionized water for 24 hours. The petroleum ether, methanolic, and aqueous extracts were concentrated individually on a water bath set at 45 ^{0}C. The extractive values of the extracts were determined using the following formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the crude extract (g)}}{\text{Weight of the powdered plant material (g)}} \text{x } 100$$

For the fractionation, one hundred grams (100 g) of the aqueous extract was suspended in water (500 ml) and successively partitioned with ethyl-acetate (5 × 500 ml) and diethyl ether (5 × 500 ml) to obtain the fractions of the various solvents. The ethyl-acetate and diethyl ether were evaporated to dryness, whereas the residual aqueous solvent was removed by placing the fraction on a water bath set at 50 °C. The fractions were kept in separately labeled tight containers before use. The solution of each fraction was prepared with distilled water for the experiment. The extractive values of each fraction were calculated based on the formula above.

Phytochemical investigations

Qualitative and quantitative phytochemical investigations were done on the crude aqueous leaves extract of *Senna alata* to test the occurrence of phytochemical constituents in the extract according to the procedure [22].

Preparation of the *Candida albicans*

The *Candida albicans* was sub-cultured on sterile Sabouraud dextrose agar (SDA) plates and incubated at room temperature (34 ± 2 ^{0}C) for 7 days before each antifungal screening.

Antifungal screening

The *Senna alata* leaves extract (petroleum ether, methanolic and aqueous) and fractions (ethyl-acetate, diethyl ether and aqueous) were investigated for antifungal effects against *Candida albicans* using the agar well diffusion method [23]. The various thin-layer chromatography (TLC) spots of the diethyl ether fraction obtained from aqueous fraction were screened for activity against the *Candida albicans* by agar overlay [24].

Agar well diffusion method

A 7-day culture of *Candida albicans* was suspended in a sterile bottle containing SDA broth. Normal saline was gradually added to it to obtain turbidity identical to Macfland standard 0.5, which corresponds to; approximately 108 cells/ml. This was subsequently diluted to produce 106 cells /ml used in the experiments. One millilitre (1 ml) of the test organism (106 cells /ml) was

inoculated into petri plates (90 mm diameter) for the antifungal susceptibility test. Then wells (6 mm diameter and 4 mm deep) were punched in the agar using a sterile cork borer. The wells were bored such that they were no closer than 15 mm to the edge of the plate and enough apart to inhibit over-lapping the inhibition zones. Some of the wells were filled up with 25 mg/ml of either one of the extracts or fractions, while some were filled up with one of the solvents as control. Adequate time was given to allow the material to diffuse considerably into each of the media. Then the plates were turned upside down and incubated at room temperature for seven days [23].

Investigation of the inhibition zones

The plates were checked for antifungal actions following the incubation period. The compounds of the discs diffused through the medium and formed a concentration gradient resulting in a clear round zone of inhibition for the test organism. Finally, the inhibition zones were measured by naked eyes with the aid of transparent scale.

Thin-layer chromatographic analysis of diethyl ether fraction obtained from aqueous leaves extract of *Senna alata*

The diethyl ether fraction (most active) of the aqueous *Senna alata* leaf extract was evaluated for the active compound using thin-layer chromatography **(**TLC) on pre-coated silica gel TLC plates (60 F254) and subsequently developed by two solvent systems; N-butanol: acetic acid: water (6:1:2) and chloroform: acetic acid (9:1). The fraction was dissolved in methanol, spotted on the pre-coated G60 F254 TLC plates, and developed in each solvent system. The various spots were detected using detecting agents, namely, iodine vapour, ammonia vapour, ultraviolet (UV) light at 366 nm, and UV in ammonia. The number of spots, spot's colours and retardation factors (Rf values) for each spot was determined. The spots obtained from the diethyl ether fraction were screened for antifungal activity against the *Candida albicans* by the agar overlay method [25].

Agar overlay method

Three millilitres (3 ml) of Sabouraud dextrose broth in a sterile capped tube was inoculated with *Candida albicans*. The inoculated bottle containing the *Candida albic*ans were incubated at room temperature for 48 hr. About 15 ml of melted SDA in three different sterile capped bottles and kept to cool at 45 ^{0}C. To each bottle containing the melted SDA, 0.8 ml of Sabouraud dextrose broth containing *Candida albicans* was pipetted and vortexed to mix the test organism. Then the content of a bottle containing the test organism was poured onto a

petri dish containing the developed TLC and control plates. A petri dish containing an unspotted TLC plate developed in the solvent system. Each Petri dish was gently tilted back and forth to distribute the agar and then allowed to gel fully evenly. The Petri dishes were then incubated at room temperature for 7 days.

Investigation of the inhibition zone

Following the incubation period, the plates were checked for antifungal effects. The compounds diffused well through the medium and formed a concentration gradient that formed a clear inhibition zone for the test organism. Finally, the inhibition zones were measured by naked eyes with transparent scale.

Chemical characterization

Column chromatographic analysis of diethyl ether fraction

The diethyl ether fraction (most active) was chromatographed over a silica gel column, mesh size; 70-230, and successively eluted with increasing polarities of n-hexane and ethyl acetate. The eluates were then applied onto a pre-coated TLC card of silica gel 60G254 (thickness 0.1mm) plates and eluted with methanol: chloroform (4:1) [25]. Eluates with the same Rf values were pooled together. Column eluted with n-hexane: ethyl acetate (40:60) yielded the most active eluate.

Gas chromatography-mass spectrometry (GC-MS) of the active spot (Spot 4)

The TLC active spot (spot 4) was analyzed using GC-MS (GCMS-QP2010PLUS) analyzer. The data were obtained on an Elite-1(100% Dimethyl polysiloxane) column (30 0.25 mm 1 µmdf). Helium (99.99%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250 ºC. GC oven temperature started at 110 ºC and holding for 2min, and it was raised to 200 ºC at the rate of 10 ºC/min, without holding. Holding was allowed at 280 ºC for 9 min with a program rate of 5 ºC/min. The injector and detector temperatures were set at 250 ºC and 280 ºC, respectively. Ion source temperature was maintained at 200 ºC. The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV, and the detector was operated in scan mode from 45-450amu (atomic mass units). A scan interval of 0.5 seconds and fragments from 45 to 450 Da was maintained. The total running time was 36 minutes.

Infrared spectroscopy of the active spot (spot 4)

The infrared spectroscopic analysis of the active spot was carried out on the KBr disc using Fourier transform (FT) Spectrophotometer (ALPHA II ,Germany).

Statistical analysis

All results are tabulated as mean ± standard error of mean (SEM). The values were analyzed by the student's T-test and F-test. The outcomes were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Extractive values of the *Senna alata* leaf extracts (petroleum ether, methanol and aqueous)

The extraction of one kilogram (1 kg) of the dried *Senna alata* leaves with different solvents (petroleum ether, methanol and water) produced yielded 150.2 g (15.02%), 104.03 g (10.40%), and 204.57 g (20.46%,). The extractive values of the extracts are displayed in Table 1.

Table 1: Extractive values of the *Senna alata* leaf extracts (petroleum ether, methanol and aqueous)

Extract	Amount (g)	Extractive values (%)
Petroleum Ether	150.21	15.02
Methanol	104.03	10.40
Aqueous	204.57	20.46

Extractive values of various fractions (ethyl-acetate, diethyl ether and aqueous) per 100 g of *Senna alata* aqueous leaf extract

The partitioning of 100 g of the aqueous *Senna alata* leaf extract with various solvents (ethylacetate, diethyl ether, and aqueous) produced 1.68 g (16.80%), 1.83 g (18.30%), and 2.45 g (24.50%) of the corresponding fractions, respectively. The extractive values of the different fractions are shown in Table 2.

Table 2: Extractive values of various fractions (ethyl-acetate, diethyl ether and aqueous) per 100 g of *Senna alata* aqueous leaf extract

Fraction	Amount (g)	Extractive values (%)
Ethyl-acetate	1.68	16.80
Diethyl ether	1.83	18.30
Aqueous	2.45	24.50

Qualitative phytochemicals investigation of the aqueous *Senna alata* leaf extract

The qualitative phytochemical determination in the aqueous *Senna alata* leaf extract displayed alkaloids, phenolic compounds, tannins, carbohydrates, reducing sugar, polyuronides, coumarins, saponins, steroids, triterpenoids, flavonoids, resins, cyanophores, cardiac glycosides and anthraquinones. However, carotenoids were absent.

Quantitative phytochemical investigation of the aqueous *Senna alata* leaf extract

The quantitative phytochemical investigation of the aqueous *Senna alata* leaf extract revealed that 10 g of the powdered leaves contained 1.27 g±0.00 (12.7%±0.01) of flavonoids, 0.64 g±0.01 (12.8%±0.01) of alkaloids, 0.004 g±0.00 (0.9%±0.00) of cyanogenic glycosides, 0.30 g±0.02 (1.48%±0.02) saponins and 0.28 g±0.01 (9.6%±0.01) of tannins. The results of the quantitative phytochemical investigation of the *Senna alata* leaf extract are shown in Table 3.

Table 3: Quantitative phytochemical analysis of the aqueous *Senna alata* leaf extract

Phytochemicals	Amount (g)	Extractive values (%)
Flavonoid	1.27 ± 0.00	12.7 ± 0.01
Saponins	0.30 ± 0,02	1.48 ± 0.02
Alkaloids	0.64 ± 0.01	12.8 ± 0.01
Cynogenic glycosides	0.004±0.00	0.90 ± 0.00
Tannins	0.28 ± 0.01	9.60 ± 0.01

Note: The values are the mean of three measurements in triplicates

Antifungal screening of the *Senna alata* leaf extracts against *Candida albicans*

All the *Senna alata* leaf extract screened (petroleum ether, methanol and aqueous) elicited an inhibitory effect against the *Candida albicans*. The aqueous extract exerted the highest

activity (13.0 ± 0.06 mm), followed by methanol extract (11.0 ± 0.12 mm). The petroleum ether extract elicited the least inhibitory action (10.0 ± 0.13 mm) against the *Candida albicans.* However, the organism was resistant to the standard antifungal agent, ketoconazole.The outcomes of the antifungal screening of petroleum ether, methanol and aqueous extracts of *Senna alata* leaves against *Candida albicans* are shown in Table 4.

Table 4: Antifungal screening of *Senna alata* leaf extracts against *Candida albicans*

Extract	**Concentration (mg/ml)**	**Zone of inhibition (mm)**
Petroleum ether	25	10.0 ± 0.13
Methanol	25	11.0 ± 0.12
Aqueous	25	13.0 ± 0.06
Ketoconazole	25	0.00 ± 0.00

Note: The values are the mean of three measurements across each zone of inhibition and in triplicates, measured in millimetre (mm). The zero values indicate no inhibition

Antifungal screening of the fractions obtained from aqueous *Senna alata* leaf extract against *Candida albicans*

The diethyl ether fraction was the most active fraction against the *Candida albicans*, with a minimum inhibition zone of 13.0 ± 0.05 mm. Besides, the ethyl-acetate fraction inhibited the test organism with a minimum zone of inhibition of 10.00 ± 0.00 mm. However, the aqueous fraction was inactive against the test organism. The results of the antifungal activities of the various fractions obtained from the aqueous *Senna alata* leafextract against *Candida albicans* are displayed in Table 5.

Table 5: Antifungal screening of the fractions obtained from aqueous *Senna alata* leaf extract against *Candida albicans*

Fraction	**Concentration (mg/ml)**	**Zone of inhibition (mm)**
Ethyl-acetate	25	10.00 ± 0.00
Diethyl ether	25	13.00 ± 0.05
Aqueous	25	0.00 ± 0.00

Note: Values are the mean of three measurements across each zone of inhibition and in triplicates, measured in millimetre (mm). The zero values indicate no inhibition

Antifungal screening of the various silica gel column eluates of diethyl ether fraction obtained from the aqueous *Senna alata* leaf extract on *Candida albicans*

The result obtained by eluting the column with n-hexane: ethyl acetate (40:60) has shown that none of the sub-fractions elicited antifungal action against the *Candida albicans.* The result of the antifungal effects of the various column eluates of the diethyl ether fraction (most effective) is shown in Table 6.

Table 6: Antifungal screening of the various silica gel column eluates of diethyl ether fraction obtained from the aqueous *Senna alata* leaf extract on *Candida albicans*

Sub-fraction	Concentration (µg/ml)	Zone of inhibition (mm)
1	200	0.00 ± 0.00
2	200	0.00 ± 0.00
3	200	0.00 ± 0.00
4	200	0.00 ± 0.00
5	200	0.00 ± 0.00
6	200	0.00 ± 0.00
7	200	0.00 ± 0.00
8	200	0.00 ± 0.00
9	200	0.00 ± 0.00
10	200	0.00 ± 0.00
11	200	0.00 ± 0.00
12	200	0.00 ± 0.00
13	200	0.00 ± 0.00
Ketoconazole	200	0.00 ± 0.00

Note: The values are the mean of three measurements across each zone of inhibition and in triplicates measured in millimetre (mm). The zero values indicate no inhibition

Antifungal screening of the various spots of sub-fraction 13 of the diethyl ether fraction from aqueous *Senna alata* leaf extract against *Candida albicans*

The outcome of TLC analysis of the sub-fraction 13 column eluates revealed three (3) spots when the plate was developed with solvent system chloroform: methanol ratio (3:1). The

antimicrobial screening of these spots for activities against *Candida albicans* revealed that none of the four spots was active against the *Candida albicans.* The result of the antifungal screening of the various spot of the sub-fraction 13 against the *Candida albicans* is shown in Table 7.

Table 7: Antifungal Screening of the various spots of sub-fraction13 from the diethyl ether fraction of aqueous *Senna alata* leaves extract against *Candida albicans*

Spots	Concentration (µg/ml)	Zone of inhibition (mm)
1	100	0.00 ± 0.00
2	100	0.00 ± 0.00
3	100	0.00 ± 0.00

Note: The values are the mean of three measurements across each inhibition zone and in triplicates measured in millimetre (mm). The zero values indicate no inhibition

Thin-layer chromatographic analysis of diethyl ether fraction from aqueous *Senna alata* leaves extract

On exposure of the TLC plate of the diethyl ether fraction, various colours were observed on the exposed TLC plate of the active diethyl ether fraction of the aqueous *Senna alata* leaf extract to varieties of detecting medium. The first spot showed bright yellow under direct sunlight. On exposure of the plate to ammonia vapour, two spots appeared as yellow and pink. Nine other spots were seen on exposing the TLC plate to iodine vapour. When checked under the UV light, the various spots produced different colours in the presence and absence of ammonia vapour.

Observation of the plates under UV light without ammonia indicated nine different spots of varying colours namely; first spot: purple (R_f=96), second spot: blue (R_f=94), third spot: light purple (R_f=80), fourth spot: orange (R_f=73), fifth spot: purple (R_f=66), sixth spot: blue-green (R_f=45), seventh spot: light purple (R_f=38), eighth spot: purple (R_f=28) and ninth spot: purplegreen (R_f=19). In contrast, on exposure of the TLC plate to UV light, in the presence of ammonia, the first spot changed from purple to blue colour, the third spot changed to pink colour; whereas 2nd, 4th, 5th, 6th, 7th, 8th, and 9th spots appeared as orange, blue-green, green, light purple, light purple, light blue and green respectively. The colour and Rf values of

the diethyl ether fraction of the aqueous *Senna alata* leaves extract after chromatographic analysis on pre-coated TLC plate and observed under various detecting mediums are displayed in Table 8.

Table 8: Rf values and colour of diethyl ether fraction from the aqueous *Senna alata* leaf extract

			Colour observed			
Spots	**R_fx 100**	**DL**	**Iodine vapour**	**Ammonia vapour**	**UV**	**UV/NH_3**
1	96	LY	Y	LY	PL	**B**
2	94	BY	Y	Y	B	P
3	80	ND	Y	LY	LP	O
4	73	ND	Y	ND	O	BG
5	66	ND	Y	ND	P	G
6	45	ND	Y	ND	BG	LP
7	38	ND	Y	ND	LP	LP
8	28	ND	Y	ND	P	LP
9	19	ND	Y	ND	PG	G

DL: Direct light, Y: Yellow, LY: Light Yellow, PL: Purple, BY: Bright Yellow, ND: not detected, G: Green, PG: purple green

Rf values and colour of fraction 13 of diethyl ether fraction obtained from crude aqueous leaf extract of *Senna alata* under different detecting medium

Thin-layer chromatographic analysis of the subfraction 13 of the diethyl ether indicated three spots with $R_f$values of 45, 54, and 93, respectively. All the three spots changed to yellow with iodine vapour. The 2nd spot changed to pink on exposure to ammonia vapour and yellow under UV light, whereas the 3rd spot changed to light blue under the UV light, as shown in Table 9.

Table 9: Rf values and colour of TLC Spots of sub-fraction 13 from diethyl ether column eluates of the aqueous *Senna alata* leaves extract under different detecting mediums

Colour Observed Under

Spots	**R_f x 100**	**DL**	**Iodine vapour**	**Ammonia vapour**	**UV**	**UV/NH_3**
1	45	ND	ND	LP	LY	LP
2	54	ND	Y	P	Y	P
3	93	ND	Y	ND	ND	B

Y: Yellow, P: Pink, LB: Light blue, ND: not detected, LB: Light pink

Infra-red spectroscopic analysis of the active column eluate of subfraction 13

The FT-IR spectrum of the 2nd spot obtained from the sub-fraction 13 of diethyl ether column eluates is indicated shown in figure 1, which shows seven vibrational bands that are allocated to their corresponding functional groups as represented in Table 10. The assigned bands are OH (3320 cm^{-1}), C-H stretching vibration (2922 cm^{-1}), conjugated C=O stretching vibration (1697 cm^{-1}), conjugated C=C stretching vibration (1648 cm^{-1}), C-H bending vibration (1514 cm^{-1}), C-O stretching vibration (1033 cm^{-1}), and C-H out of plane bending vibration (670 cm^{1}) bonded to a carbon-carbon double bond (C=C).

Table 10: Infra-red spectroscopic analysis of the active column eluate of subfraction 13

Bands	**Wave number(cm^{-1})**	**Assignment**
1	3320	O-H Stretching
2	2922	C-H Stretching
3	1697	C=O Stretching
5	1514	C-H Bending
6	1033	C-O Stretching
7	670	C-H Bending

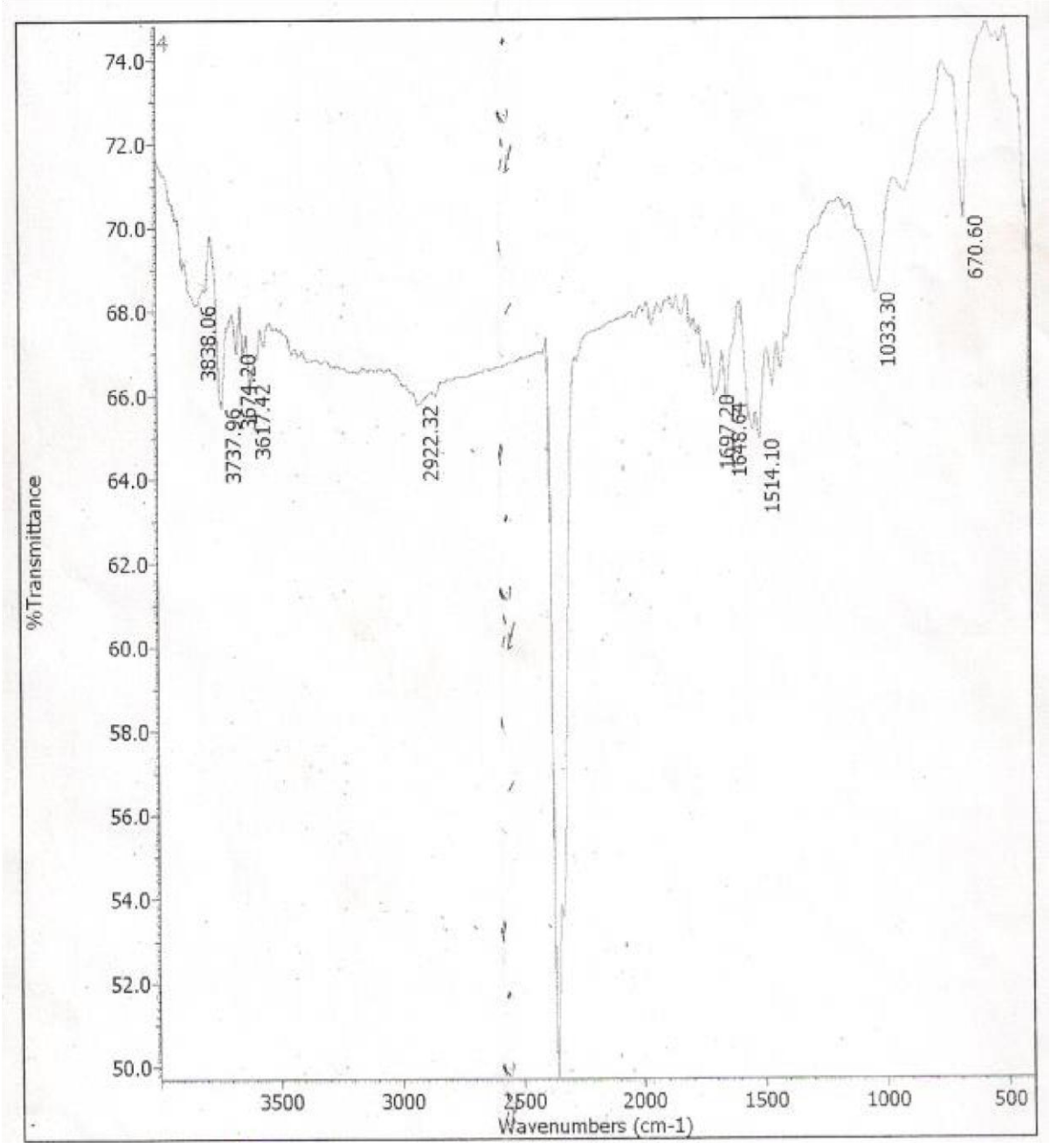

Figure 1: Infrared spectrum of Spot 4 of fraction 13 obtained from diethyl ether column eluates

Gas chromatography-mass spectrometry (GC-MS) of spot 2 from subfraction 13 column of the diethyl ether

Total ion chromatogram (TIC) of the subfraction 13 2nd spot from the diethyl ether column eluates revealed in Figure 2 has shown that peak 3 as the clearest peak eluted at a retention time of 22.10 minutes is part of the 2nd spot from the subfraction 13. Other TIC peaks could be the possible impure compounds present in the elute. The electron impact mass spectrometry (EIMS) spectrum of the spot 4 of sub-fraction 13 revealed clear peaks as

represented in figure 3 and Table 11 that revealed a molecular ion peak at *m/z* 296$[M]^+$ with the following fragment ion peaks at m/z 264 $[C_{19}H_{36}]^+$, 222 $[C_{16}H_{30}]^+$, 180 $[C_{13}H_{24}]^+$, 137 $[C_{12}H_{17}]^+$, 112 $[C_8H_{16}]^+$, 98 $[C_7H_{12}]^+$, 84 $[C_6H_{12}]^+$, 83 $[C_6H_{11}]^+$, 69 $[C_5H_9]^+$, 55 $[C_4H_7]^+$, 41 $[C_3H_5]^+$ and 40 $[C_3H_4]^+$. The fragment ion at *m/z* 264 in relation to $C_{19}H_{36}^+$ was obtained from the loss of two oxygen atoms due to the fragmentation of the molecular ion at *m/z* 296 $[M]^+$. The fragment ion at *m/z* 84 $[C_6H_{12}]^+$ revealed the loss of 180 mass $[C_{13}H_{24}]^+$ ion. The fragment ion loss at *m/z* 137 is associated with a loss of a carbon-carbon double bond (C=C), whereas the fragment ion at *m/z* 55 represents the base peak ion.

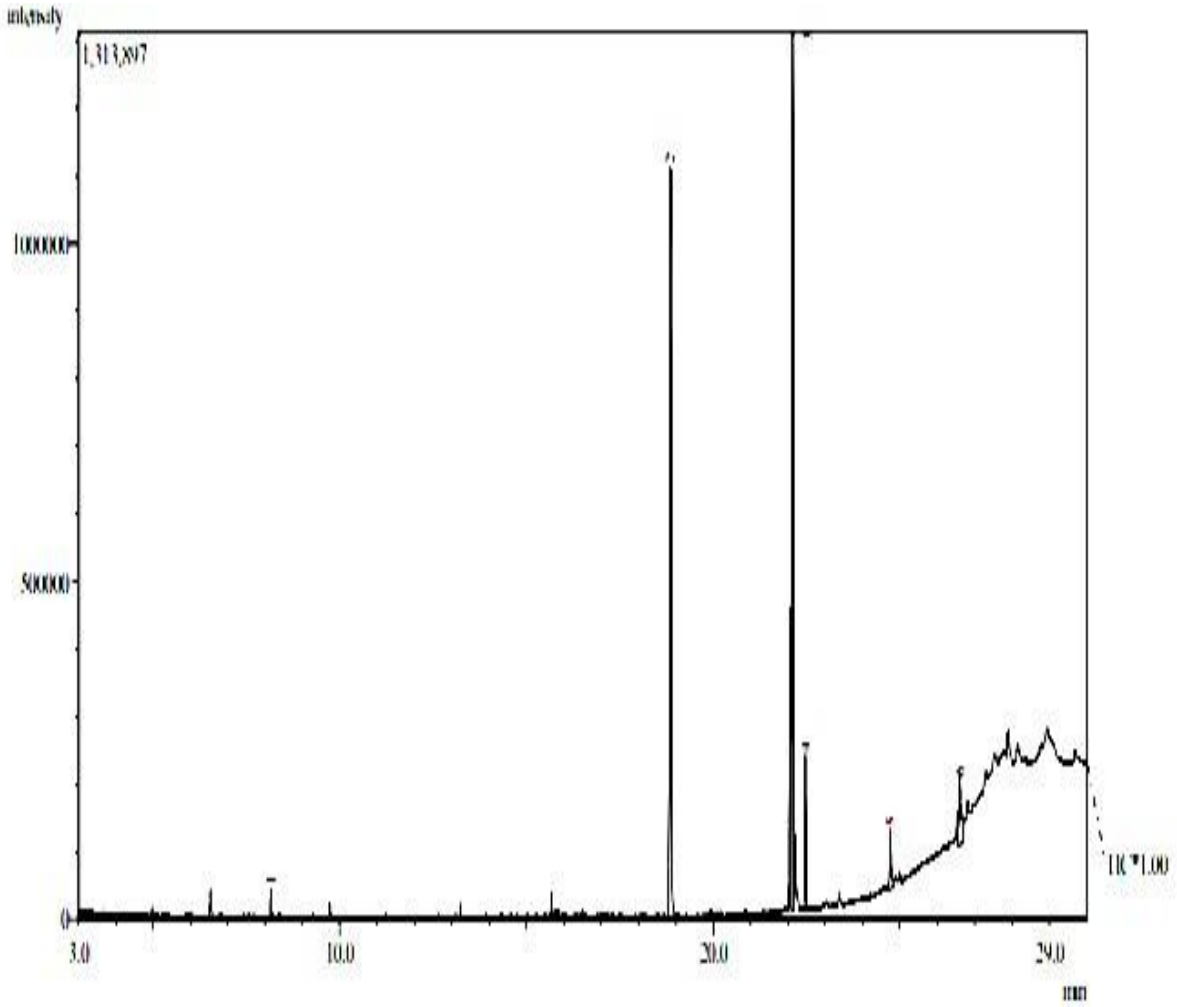

Figure 2: Gas-Chromatography Chromatogram of the diethyl ether subfraction 13 of Spot 2

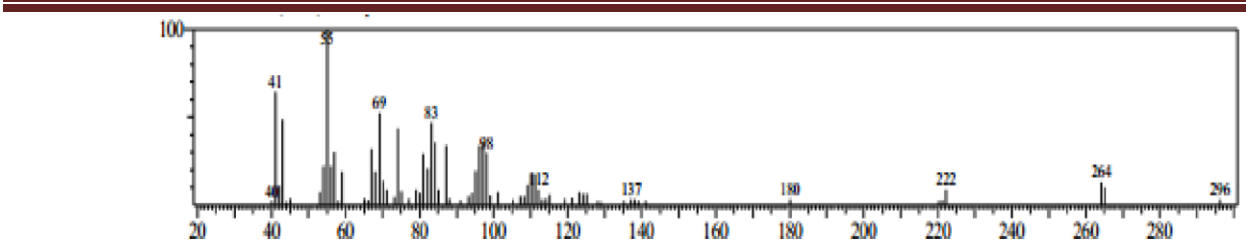

Figure 3: Electron impact mass spectrometry (EIMS) spectrum of Spot 2 of fraction 13 obtained from diethyl ether column eluates.

Table 11: Mass spectrum of spot 2 of sub-fraction 13 obtained from diethyl ether column eluates of *Senna alata* aqueous leaf extract

m/z	**Percentage relative abundance**
296	4.32
264	11.35
222	8.11
180	2.70
137	3.78
112	16.22
98	32.43
84	37.84
83	46.45
69	53.51
55	100.00
41	64.87
40	2.16

Based on this research, water produced the highest yield, which was almost twice that of methanol and one and a half times that of petroleum ether, an indication that it could have the highest phytochemicals which concurs with a previous research [29]. Interestingly, the antifungal activity of the aqueous *Senna alata* leaf extract against drug-resistant *Candida albicans* in this work agrees with the previous findings [30]. However, previous researches have shown that the aqueous *Senna alata* leaves extract was inactive against the *Candida albicans*[18,31]. Besides, the inhibitory action of the methanol extract of *Senna alata* against drug-resistant *Candida albicans* in the current work agrees with that of the previous studies [30-33]. Similarly, the antifungal action elicited by the petroleum ether extract against the

drug-resistant *Candida albicans* concurs with previous research [19]. The varying degrees of the inhibitory effect exerted by the various *Senna alata* extracts in this study could be related to the amount of the phytocompounds due to their variation in the degree of solubility in solvents with different polarities [34, 35].

In bioassay-guided fractionation, various fractions are evaluated for potential pharmacological actions. The most effective fractions are subsequently fractionated and further investigated to discover a lead compound [36]. In this work, the antimicrobial action exhibited by the diethyl ether fraction against the *Candida albicans* could be associated with the higher contents of the phytocompounds as observed by its higher extractive yield than the ethyl-acetate fraction. A previous study has shown the capability of ethyl-acetate fraction against the *Candida albicans* [37].

The analysis of plants bioactive agents is useful in determining the class of the plants' phytocomponents and their amount, which could serve as a basis to isolate bioactive compounds [38]. In this research, the aqueous *Senna alata* leaves extract indicated saponins, alkaloids, emodin, tannins, steroids, anthraquinones, and flavonoids responsible for the antimicrobial ability, which concurs with the previous investigations [39,40]. In general, tannins, phenols, saponins, steroids, alkaloids, and flavonoids possess anti-infective actions through different biological mechanism [41-43]. For instance, flavonoids interfere with microbial enzymatic actions, inhibit cell wall synthesis and destabilize microbial cell membrane [44-46]. The tannins prevent microbial adhesions and fungal enzymatic reactions [41,46]. The anthraquinone derivatives also exhibit biological activities against fungal infections [47-49]. The saponins also serve as a major antifungal secondary metabolite that changes cell wall permeability, enhances the influx of toxic agents into the cell, and facilitates the leakages of vital cell components [50,51]. As such, the presence of these secondary metabolites in the *Senna alata* could be associated with its antimicrobial actions.

The loss of activity of the subfraction 13 of the diethyl ether fraction against the drug-resistant *Candida albicans* in the current work could be due to the separation of the active components that probably act synergistically or due to resistance by the test organism. The bioactive spot (Spot 2) of subfraction 13 from the diethyl ether fraction possessed a hydroxyl group in its structure as shown in its IR spectrum assignable to the vibrational band at 3320 cm^{-1}, which is broad and of low intensity. This is typical of the –OH group found in fatty acids, and it belongs to –OH bonded to a carboxyl group carbon atom. The presence of a carboxyl group in the structure of the 2nd spot of the subfraction 13 has been confirmed by the

GC-MS spectral data shown. The loss of two oxygen atoms from the fragmentation of the molecular ion at *m/z* 296 to form the fragment ion peak at *m/z* 264 showed that this loss of oxygen atom arises from a carboxyl group, which is typical of fatty acids methyl ester [52].

Besides, the FT-IR spectrum of the 2nd spot of subfraction 13 indicated carbon-carbon double bond (C=C) at 1684 cm^{-1} which was confirmed in the GC-MS spectral data because the fragment ion at *m/z* 137 is accompanied by a loss of carbon-carbon double bond (C=C) to form the fragment ion at *m/z* 112. The C=C bond orientation is *cis* configuration which was confirmed by C-H out-of-plane bending vibration (670 cm^{-1}) of an olefinic bond due to the absence of the bending vibration at 950-970 cm^{-1}, which is a typical feature of *trans* configuration in the IR spectrum of the 2nd spot from the subfraction 13. Additionally, the CH bending vibration at 1514 cm^{-1} is due to the terminal (CH_3) group in the eluate, which was confirmed by the GC-MS spectral data, because the fragment ion at *m/z* 83 loses a methyl group and one hydrogen atom to form the fragment ion at *m/z* 69, a typical characteristic of unsaturated fatty acid with a methyl group at the terminal end [52].

The IR spectra information of the 2nd spot from the sub-fraction 13 concurs with that of *cis*Octadecenoic acid methyl ester [53]. The whole fragment ions in the GC-MS spectra data of the 2n spot, including the molecular ion and base peak ion, corresponds with the information for methyl 9-octadecenoate [54]. Hence, the data showed in the current investigation and that obtained from the literature by comparison with the library search outcome, the 2nd spot of the subfraction 13 was suggested as 9-octadecenoic acid methyl ester (E) with the molecular formula ($C_{19}H_{35}O_2$) based on the data from its molecular ion at *m/z* 296 [55]. In this experiment, the main fatty acids in the *Senna alata* are oleic acids [25], which shows the potential presence of fatty acids in the *Senna alata* leaves. Therefore, the antimicrobial effects of the *Senna alata* could be related to the presence of the fatty acid methyl ester on the basis of the library search and spectral analysis [55]. The long-chain unsaturated fatty acids, such as oleic acids, are efficacious antimicrobial agents [56-58]. These information could serve as the basis for the observed antimicrobial activity against the *Candida albicans*.

CONCLUSION

This research has revealed that the leaves extract of *Senna alata* have antimicrobial actions against *Candida albicans* and serve as a source for the effective antifungal compound. Besides, the possible antimicrobial activity of the *Senna alata* leaves might be associated with

the presence of 9-Octadecenoic acid methyl ester (E), which may serve as a novel compound for further investigation to develop new bioactive agents against drug-resistant fungal infections. It is recommended for further spectroscopic studies such as Nuclear magnetic resonance (NMR) to be carried out to elucidate the structure compound identified. Besides, more biological screening and safety assessment on the identified compound should be conducted to elucidate its mechanisms of antifungal activities and safety profile.

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