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Phytoconstituents and antidermatophytic activity of crude extracts of Senna occidentalis

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Abstract

Dermatophytes are one of the major aetiologic agents of cutaneous mycoses Senna occidentalis is among the plants used in traditional herbal medicine in treating fungal skin infections and it is shown from literature to contain phytochemicals which are attributed to its antidermatophytic activity. This work is aimed at determining the Phytoconstituents and antidermatophytic activity of leaves and seeds crude extracts of the Senna occidentalis plant The study is a qualitative study that determines the phytoconstituents and antidermatophytic activity of the plant extracts on some clinical dermatophyte isolates. The plant parts were sampled and were used to obtain aqueous and n-hexane extracts using distilled water and n-hexane as extracting solvents respectively. Phytochemical analysis was done on the extracts to determine the presence of secondary metabolites. The antidermatophytic activity of the extracts on clinical dermatophytes isolates was determined using poisoned food technique. Aqueous extraction gave higher percentage extraction yield than n-hexane extract . All extracts contain secondary metabolites and the extracts showed varying degree of percentage growth inhibition on the isolates. Phytochemical screening of the leaves and seeds extracts of Senna occidentalis revealed the presence of alkaloids, saponins, tannins and other phytoconstituents. The Senna occidentalis leaves and seeds extracts showed growth inhibition percentage (I) ranging between 9% to 39.8% for n-hexane leaf extract, 1.3% to 52.6% for aqueous leaf extract, 2.6% to 57.2% for n-hexane seed extract and 12.8% to 61.1% for aqueous seed extract. Senna occidentalis leaves and seeds extract have shown varying potential in inhibiting dermatophyte growth with no extract having 100% inhibition percentage on all the tested dermatophytes.

Keywords: antidermatophytic, extract, Senna occidentalis, Phytoconstituents.

INTRODUCTION

Man has been using medicine derived from Herbs for hundreds of years as a source of treatment for himself, his domestic animals and plants prior to 20th century making it a major aspect of socio-cultural heritage (Ogunkunle and Ladejobi, 2006; Osamene *et al.*, 2011; Nwauzoma and Dappa, 2013). The revival of herbal medicine at the close end of the 20th century is because of the rising cost of imported medication to the extent that government imported medication cannot meet the demands of the people. So also, the scarcity and cost of commodities used to manufacture drugs locally have made modern medicine too expensive for the common man to afford (Ogunkunle and Ladejobi, 2006). Since dependence of medicines derived from indigenous plants is predominant in developing countries (Adelanwa and Tijjani, 2013), this shows the need that we continually evaluate and develop our indigenous plant resource for the improvement and sustenance of

our health care delivery system (Ogunkunle and Ladejobi, 2006: Mohamed et al., 2020) since indigenous medicinal plants form an important component of the natural wealth and culture of Nigeria (Adelanwa and Tijjani, 2013). Skin mycoses is one of the most frequent fungal infections worldwide caused bγ both dermatophytes and some non-dermatophyte fungi (Arava et al. 2021). It is estimated that about 20-25% of the world's population has skin mycoses (Havlickova et al., 2008; Kaul et al., 2017). Effective antifungal drugs are inaccessible to large numbers of the global population. Where available, prices are often costly for majority of the population, making them in-accessible to many people (Kneale et al., 2014). Alternative to conventional drugs could be obtained through exploration of medicinal plants. Senna occidentalis is a flowering plant of the Leguminosae family, it is commonly known as Coffee senna. In Nigeria it is known as 'Rai Dore', Hausa, 'Aborere' Yoruba

UJMR, Vol. 8 No. 1, June, 2023, pp 152-160. and 'Okamo', Igbo (Ogunkunle and Ladejobi, 2006; Abubakar et al., 2007). Preparations made from parts of this plant locally to treat Eczema and other skin defects caused by fungal infections by scrubbing the affected areas with the leaves and allowing it to dry on the affected areas. This process is repeated for days until the infection disappears (Ogunkunle and Ladejobi, 2006). Phytochemical analysis using different extraction solvents such as water, butanol, hexane, acetone, ethanol, and methanol shows the presence of secondary metabolites in this plant (Aja et al., 2017) such as flavonoids saponins, tannins, terpenes, and glycosides (Nde et al., 2022). Pharmacological investigations showed that Senna occidentalis plant extracts from leaf and other parts have Broad spectrum antibacterial, antimalarial, and antifungal activity (Sule et al., 2010). It also cures cough and indigestion (Vipul and Anjana, 2011).

The aim of this research was to determine the Phytoconstituents and antidermatophytic activity of leaves and seeds crude extracts of *Senna occidentalis*.

MATERIALS AND METHODS

Collection and Identification of Plant Materials Whole plant of *Senna occidentalis* was obtained from the bushy parts of Samaru metropolis and was taken to the Herbarium of department of Botany, ABU Zaria where it was identified and was given a voucher number 06868.

Preparation of Plant Extracts

The leaves and seeds samples were cleaned and washed using tap water and were dried under shade until they are completely dried, the samples were pulverized in to powdered and form were stored in clean airtight containers (Delahaye *et al.*, 2009; Abdullahi-Gero *et al.*, 2014).

Aqueous Extraction

Distilled water was used as extracting solvent. Two hundred grams of each of the plant's materials (leaves or seeds) was placed in a clean conical flask and about 500 mL of distilled water was poured to completely immerse the plant materials. It was stoppered using cork stopper and the set up was placed on an electric shaker for 8 hours after which it was allowed to stand over night the set up was filtered using a clean Muslin cloth and then through filter paper. Additional distilled water was used to rinse the residue to ensure complete extraction and the filtrate was poured into a porcelain bowl and was placed on a water bath at 44-50°C until the solvent evaporates completely. The residual extracts were stored in a clean container with (Samuel et al., 2013; Abubakar and lid Haque, 2020).

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N-hexane Extraction

The plant materials were placed in a Soxhlet extractor and N-hexane was used as the extracting solvent. The filtrate obtained from the extractor was then poured in to a porcelain bowl and placed on a water bath at $45^{\circ}C-50^{\circ}C$ until the residual solvent evaporates completely (Samuel *et al.*, 2013; Abubakar and Haque,2020). The 4 extracts obtained were stored in a clean container with lid.

Percentage yield of extraction

This was calculated for each extract using the following formula.

Percentage yield $= \frac{weight of concentrated crude extract (g)}{weight of the dried crushed plant sample used (g)} X 100$ (Anokwuru *et al.*, 2011; Dhanani *et al.*, 2013; Daskum *et al.*, 2019 and Tochukwu *et al.*, 2021) Phytochemical screening

The extracts obtained were subjected to various phytochemical analysis tests to determine the secondary metabolites present. The procedure used was as described by Sofowora (1993) and Trease and Evans (1989).

Preparation of test concentrations of the extracts

Preparation of aqueous extract concentrations Two and a half grams (2.5g) of each extract was dissolved in 10 mL of sterile distilled water in a sterile container, 5 mL from this stock solution was serially diluted using doubling dilution up to 4 times to obtain a total of five concentrations namely 250.00 mg/mL, 125.00 mg/mL, 62.50 mg/mL, 31.25 mg/mL, and 15.63 mg/mL of the extract. About 5mL of each of the different concentrations were mixed with 145 mL of molten SDA at a temperature of 45°C and was poured into sterile Petri dishes and allowed to solidify (Kumar and Tyagi, 2013; Qadoos *et al.*, 2016). Duplicate plates were used for each isolate for a given extract.

Preparation of N-hexane extracts concentration

Two and a half grams of each extract was dissolved in 10 mL of the mixture of 1:4 n-hexane tween 80 solution in a sterile container and 5 mL from this stock solution was serially diluted using doubling dilution up to 4 times in the same mixture of 1:4 n-hexane and tween 80 solution to obtain a total of five concentrations given as 250.00 mg/mL, 125.00 mg/mL, 62.50 mg/mL, 31.25 mg/mL, and 15.63 mg/mL. About 5 mL of each of the different concentrations were mixed with 145 mL of molten SDA at a temperature of about 45°C and was poured into sterile Petri dishes and was allowed to solidify. This procedure was repeated until duplicate plates were obtained for all the isolates for a given extract (Fayaz et al., 2017).

UJMR, Vol. 8 No. 1, June, 2023, pp 152-160. Control plates preparation

The control plates were prepared for each organism and for each extract. The controls were:

SDA plates containing neither extracts nor solvent: these are used as negative control and were inoculated with the isolates.

SDA containing n-hexane and tween 80 only: 5ml of N-hexane tween 80 solution was mixed with 145 mL of Molten SDA at about 45°C and was poured into Petri dishes and allowed to solidify. This was inoculated with the isolates and growth was compared to positive control to see the effect of N-Hexane and tween 80 on growth of isolates.

SDA containing ketoconazole: Two hundred milligram of ketoconazole was dissolved in 10 mL of sterile distilled water and was mixed with 90 mL of molten SDA and was poured into sterile Petri dishes and was allowed to solidify. Duplicate pates were used for all the test organisms. This was inoculated with the isolates (Kumar and Tyagi. 2013). This served as positive control.

Antidermatophytic Susceptibility Testing

The clinical isolates used were dermatophytes namely Trichophyton verrucosum, Trichophyton rubrum, Microsporum species II, Trichophyton species I and Microsporum species I which are isolated from samples collected from male children attending Almajiri Islamiyya schools in Samaru district, Zaria- Kaduna state, Nigeria. By applying the procedure of poisoned food technique, the inoculum size used for susceptibility test was a 7 mm disc of actively growing (6-7 days) culture of the dermatophytes . The labeled media plates prepared were

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inoculated by placing the agar disc of the isolates aseptically upside down at the center of each of the SDA plates containing the varying concentrations of the extracts and were labeled accordingly for each isolate (Kumar and Tyagi, 2013; Qadoos et al., 2016). The plates were incubated at room temperature until the organisms on the control plates of SDA only filled the plate completely or until the end of the test at day 7. The plates were observed daily. Mutual growth diameter for each organism were measured and recorded (Kumar and Tyagi, 2013). The growth diameter was also noted for the other control plates at the same time interval. The antifungal activity was evaluated by measuring the relative growth inhibition in plates with extracts and growth in control plates. The percentage growth inhibition over control was determined by using the formula.

 $I = \frac{c-T}{c} \times 100$ Where I for inhibition percentage C for colony diameter (mm) in control T for is colony diameter (mm) in treatment (Kumar and Tyagi, 2013; Qadoos et al., 2016; Fayaz et al., 2017).

RESULTS

Table 1 shows the nature of extracts and percentage extraction yield of solvents for each plant sample. All N-hexane extracts of the plant samples were green in colour. All Aqueous extracts of the plant samples were brown in colour and the percentage yield for aqueous leaf and seed extracts of Senna occidentalis were the highest compared to their N-Hexane counter parts.

Table	1: Extracts and per	rcentage extraction	yield	of solv	vents on	samp	oles of Senna	occidentalis.
		_			-	-	-	

Plant	Extracts	Colour of Extract	Nature of extract	Percentage extraction yield (%)
Senna occidentalis	Aqueous leaf	Deep brown	Sticky matter	15.75
	N-Hexane leaf	Green	Clumpy matter	6.33
	Aqueous seed	Brown	Chunks of matter	14.11
	N-Hexane seed	Green	Oily liquid	5.23

Table 2 shows the phytochemical constituents of Senna occidentalis extracts. The extracts of the leaves show the presence of alkaloids, saponins, tannins and other phytoconstituents, while alkaloids, saponins and tannins were not detected in n-Hexane extract of the leaves. The Aqueous extract of the seeds had a similar profile as aqueous extract of the leaves, while

the n-Hexane extract had alkaloids but saponins and tannins were not detected. The aqueous extracts of Senna occidentalis leaves and seeds both showed higher phytoconstituents compared to n-hexane extract of both leaves and seeds of the plant. Anthraguinones was not detected any of the extracts.

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Phytochemical	Senna occidentalis			
constituent	Leaves		Seeds	
	Aqueous extract	N-hexane extract	Aqueous extract	n-hexane extract
Alkaloids	+	-	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	-	+	-
Phenolic compounds	+	+	+	+
Tannins	+	-	+	-
Steroids	+	+	+	+
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Anthraquinones	-	-	-	-

Table 2 : Phytochemical Constituents of Leaves and Seeds of Aqueous and N-Hexane extracts of Senna occidentalis

Key + present and - absent

Table 3 presents the mean of average growth diameter of each of the extracts at all five concentrations as well as the calculated standard error of the averages. M. species II and T. species I have the highest average growth diameter at all concentrations for n-hexane leaf extract this is indicated by the standard error value of zero. T. rubrum ,T. verrucosum and M. species II all have a standard error value of more than zero due to variability in the average growth diameter at the different extract concentrations. for aqueous leaf extract, M.

species II is the only dermatophyte with standard error value of zero while all others have a value of greater than one. for n-hexane seed extract, M. species I has the lowest value of less than one while all others have a value of more than 1 indicating the variability in the average growth the different diameter at extract concentrations. for aqueous seed extract only T. verrucosum and T. rubrum have a standard error value of more than one all others have a value of zero.

Table 3: Mean average growth diameter and standard error of Senna occidentalis extracts on the dermatophyte isolates.

Organisms	Senna occidentalis extracts / Mean(mm) ± SE						
	n-hexane leaf	-hexane leaf Aqueous leaf n-hexane seed		Aqueous seed			
T. verrucosum	40.4 ± 0.68	29 ± 2.71	36.4 ± 3.97	25.6 ± 3.99			
T. rubrum	29.4 ± 2.92	29.7 ± 3.48	31.9 ± 2.43	29.4 ± 2.13			
M. species II	83 ± 0	83 ± 0	76.1 ± 3.22	83 ± 0			
T. species I	83 ± 0	80.7 ± 2.30	68.6 ± 7.28	83 ± 0			
M. species I	74 ± 4.06	80.1 ± 2.90	74.7 ± 0.44	83 ± 0			

Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: KEY: T. verrucosum: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I

Table 4 shows the antidermatophytic activity of n-Hexane extract of Senna occidentalis leaf on the dermatophyte isolates. *Microsporum species* Il was inhibited at a higher concentration. Microsporum species II and Trichophyton species I were not inhibited at all concentrations. Trichophyton rubrum was inhibited with increase in concentration while Trichophyton verrucosum showed an indefinite pattern of inhibition at all concentrations.

Table 4: Antidermatophytic activity of n-Hexane extract of Senna occidentalis leaf on the dermatophyte isolates

Extract (mg/mL)	concentration	Dermatophytes /Percentage growth inhibition (%)						
		T. verrucosum	T. rubrum	М.	species	T. species	M. species I	
				II		1		
0		0	0	0		0	0	
15.63		15.8	10.3	0		0	0	
31.25		9.5	9	0		0	19.9	
62.50		15.8	24.4	0		0	8.4	
125.00		17.9	29.5	0		0	22.9	
250.00		15.8	50	0		0	39.8	

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KEY: T. verrucosum: Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I Table 5 shows the antidermatophytic activity of aqueous extract of Senna occidentalis leaf on dermatophyte isolates. Trichophyton the verrucosum and Trichophyton rubrum were inhibited and the higher the concentration, the

higher the inhibition. Microsporum species II was not inhibited at all concentrations. While Trichophyton species I and Microsporum species I was inhibited at a 250.00 mg/mL concentration of extract.

Table 5: Antidermatophytic activity of Aqueous extract of Senna occidentalis leaf on dermatophyte isolates

Extract (mg/mL)	concentration	Dermatophytes	ermatophytes /Percentage growth inhibition (%)						
		T. verrucosum	T. rubrum	M. speciesll	T. speciesl	M. speciesl			
0		0	0	0	0	0			
15.63		35.8	9	0	0	0			
31.25		46.3	1.3	0	0	0			
62.50		48.4	26.9	0	0	0			
125.00		46.3	29.5	0	0	0			
250.00		17.9	52.6	0	13.9	12.5			

Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: KEY: T. verrucosum: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I

Table 6 shows the antidermatophytic activity of n-Hexane extract of Senna occidentalis seeds on dermatophyte isolates. the Trichophyton verrucosum and Trichophyton rubrum were inhibited at all concentrations. So also, the higher the concentration the higher the

inhibition. Microsporum species I, Trichophyton species I and Microsporum species II were not inhibited at lower concentrations but were inhibited at 62.50mg/Ml,125.00mg/mL and 250.00mg/ concentrations. The higher the concentration the higher the inhibition.

Table 6: Antidermatophytic activity of n-Hexane extract of Senna occidentalis seeds on dermatophyte isolates

Extract (mg/mL)	concentration	Dermatophytes /Percentage growth inhibition (%)							
		T. verrucosum	T. rubrum	M. species II	T. species I	M. species I			
0		0	0	0	0	0			
15.63		10.5	2.6	0	0	0			
31.25		11.6	10.3	0	0	0			
62.50		16.8	20.5	4.8	10.8	4.2			
125.00		22.1	17.9	15.7	33.1	24.7			
250.00		55.8	39.7	18.7	42.8	57.2			

KEY: T. verrucosum: Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I

Table 7 shows the antidermatophytic activity of Aqueous extract of Senna occidentalis seed on the dermatophyte isolates. *Microsporum species* II, Trichophyton species I and Microsporum I were not inhibited at species all concentrations. Trichophyton verrucosum was inhibited most by this extract the higher the

concentration the higher the inhibition. Trichophyton rubrum was also inhibited by the extract, the higher the concentration, the higher the inhibition with exception of 125.00 mg/mL concentration where it is lower than 62.50 mg/mL concentration inhibition.

Table 7: Antidermatophytic activity of Aqueous extract of Senna occidentalis seed on the dermatophyte isolates.

Extract (mg/mL)	concentration	Dermatophytes /Percentage growth inhibition (%)						
		T. verrucosum	T. rubrum	M. speciesII	T. speciesl	M. speciesl		
0		0	0	0	0	0		
15.63		54.7	12.8	0	0	0		
31.25		53.7	17.9	0	0	0		
62.50		61.1	24.4	0	0	0		
125.00		47.4	23.1	0	0	0		
250.00		13.7	44.9	0	0	0		

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UJMR, Vol. 8 No. 1, June, 2023, pp 152-160. KEY: T. verrucosum: Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I Figure 1 shows the antidermatophytic activity of ketoconazole which is the positive control. Trichophyton verrucosum was completely inhibited Trichophyton rubrum and .

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Trichophyton species I were inhibited above 75% while the Microsporum species were inhibited above 90%.



Figure 1: Antidermatophytic activity of Ketoconazole against the dermatophyte isolates. KEY: T. verrucosum: Trichophyton verrucosum, T. rubrum: Trichophyton rubrum, M. species II: Microsporum species II, T. species I: Trichophyton species I and M. species I: Microsporum species I

DISCUSSION

Aqueous extract of Senna occidentalis leaves and seeds revealed the presence of alkaloids, saponins, tannins and other phytoconstituents with the absence of anthraguinones. Alkaloids, Saponins and Tannins were not detected in n-Hexane extracts of the leaves, but it contains all other components present in the aqueous extract. A study by Daskum et al., (2019) reported the presence of anthraquinones, phenols, tannins, alkaloids, and flavonoids in the extract and Ettu et al., (2011) revealed the presence of anthraquinones in the n-hexane extract of Senna occidentalis leaves. N-hexane extract of the seeds differs from that of the leaves by the presence of alkaloids in the extract. The N-hexane extract of both leaves and seeds have a high growth inhibition percentage (I) on some of the dermatophytes of above 50%. Nnagbo and Anvian, (2021) confirmed the antidermatophytic activity of n-hexane extracts of Senna occidentalis leaves. The observance of some degree of growth inhibition in some of the extracts on the dermatophytes might be attributed to the presence of secondary metabolites which are reported to have antidermatophytic activity (Chahal et al., 2021). Saponin which is an effective antifungal agent is confirmed to be an active antifungal agent due to it being a special class of glycoside that have soapy characteristics (Salhi et al., 2017). They are also reported to be responsible for the leakage of certain enzymes and proteins from the cell wall by their ability to perforate

lipid bilayers and increase the permeability of the cell membrane allowing transport of molecules that will otherwise be excluded (Chepkwony et al., 2021).). Flavonoids were reported to have showed good antidermatophytic activity which is related to fungistatic and fungicidal activity of secondary metabolites on the organisms . Also, terpenoids have been associated with the weakening of the membrane tissue leading to the dissolution of the fungal cell wall (Chepkwony et al., 2021). The extracts also contain phenols and tannins proteins which denature can at low concentrations and precipitate proteins at higher concentrations (Wang *et al.*,2020). Crude plant extracts have been reported from literature to have antimicrobial activity against filamentous fungi including dermatophytes. The mechanism of action of these extracts is diverse. Some research reports the extracts interference with the cell wall and cell membrane of the fungi leading to cell lysis and death (Gonelimali et al., 2018; Kha and Le, 2020). Other studies suggests that the extract may inhibit fungal growth by interfering with metabolic pathways (Kha and Le, 2020; Savarirajan *et al.*,2021; Ivanov *et al.*, 2022). The antifungal activity of crude plant extracts depends on many factors, and it does not always increase with an increase in extract concentration for all plants, it depends on the type of plant and the type of fungi being tested. Some plants have a higher antidermatophytic activity due to increase in concentration of bioactive compounds (Kha and

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Le, 2020). Also, chemical reactions between the components of the extract might affect its antidermatophytic activity at higher extract concentrations. In a study by Lv et al., (2022) they attributed the drop in growth inhibition at higher extract concentration to the possibility that the effective antifungal component might have precipitated with the extract at higher concentration. The extracts may have different spectra of antidermatophytic activity due to fact that there is a difference in solubility or insolubility of the active compounds in the solvent used for extraction (Fayaz et al., 2017). The mode of action of phytochemicals such as flavonoids, steroids and alkaloids against fungi has been found to be through disruption of integrity of cell wall, cytoplasmic membrane damage, ROS accumulation, mitochondrial dysfunction, and biofilm inhibition among others (Lee and Lee,2015; Makhuvele et al.,2020). Trichophyton rubrum was found to be susceptible to n-hexane extract of Senna occidentalis in a study using microdilution technique by Nnagbo and Anyian, (2021). A study by Savarirajan et al., (2021) showed that ethanolic extract of Senna occidentalis seeds have strong antidermatophytic activity of 100% inhibition percentage (I) on Microsporum and Trichophyton species using poisoned food method. Tamasi et al., (2021) in another study using well diffusion method showed the antidermatophytic activity of methanol and ethyl acetate extract of Senna occidentalis plant against Trichophyton rubrum. In this study, the raw data obtained is not the determinant of the potency of the extract, it is the calculated

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inhibition growth percentage (I) that determines the potency of an extract. standard error is used as a statistical tool in this research to show how accurate the mean of the measured growth inhibition from those measurements is likely to be compared to the true population mean. Smaller standard error value indicates that the average growth inhibition value is similar for all the extract concentrations while larger standard error value indicates a larger difference in the average growth inhibition value for all extract concentrations for a given dermatophyte. The Senna occidentalis leaves and seeds extracts showed calculated growth inhibition percentage (I) ranging between 9% to 39.8% for n-hexane leaf extract, 1.3% to 52.6% for aqueous leaf extract, 2.6% to 57.2% for n-hexane seed extract and 12.8% to 61.1% for aqueous seed extract but none of them has reached the recommended threshold of an inhibition percentage (I) of 100% (Savarirajan et al., 2021) for its result to be analyzed to know whether it is statistically significant or not. Based on the information presented, it confirms the traditional expectation that medicinal plants such as Senna parts can be used for local occidentalis medicinal purposes (Aremu et al., 2016).

CONCLUSION

The phytochemical screening of Senna occidentalis leaves and seeds crude extracts in this study revealed the presence of saponins, flavonoids, tannins, and others as secondary metabolites . The extracts exhibited calculated inhibition percentage (I) of below 100% on the dermatophyte isolates

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