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ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF *SENNA SINGUEANA* (DELILE) LOCK (FABACEAE)

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ABSTRACT

Senna singueana, locally called ''rumfu'' in Hausa, is used traditionally for venereal diseases, cough, wounds, eye infections, etc. This study aims to justify the traditional uses of *Senna singueana* leaves in microbial infections. Plant materials were collected, authenticated, powdered and extracted successively using n- hexane, ethyl acetate and methanol. Preliminary phytochemical screenings were carried out using standard methods. The antimicrobial activity was assessed using the agar well diffusion method. Microorganisms used were; gram-positive bacteria (*Staphylococcus aureus, Streptococcus faecalis,),* gram-negative bacteria *(Escherichia. coli, Pseudomonas aeruginosa, Shigella dysenteriae Campylobacter jejuni, Salmonella typhi, Neiseria gonorrhea, Proteus mirabilis)* and fungi *(Candida albican, Candida tropicalis and Candida Krusei)*. Ciprofloxacin and Fluconazole were used as standards. Hexane, ethyl acetate and, Methanol extracts were obtained. The presence of flavonoids, alkaloids, triterpenes, and glycosides were detected. The ethyl acetate extract (EE) was found to be the most effective, showing the highest zone of inhibition against *E. coli, Strep. faecalis*, and *Staph. aureus*. *N. gonoreae, P. aeruginosa, C. jejuni and P. mirabilis* were resistant to all extracts. The Minimum Inhibitory Concentrations (MIC) values of EE were from 1.25 mg/ml to 5 mg/ml and Minimum Bactericidal/fungicidal Concentrations (MBC/MFC) values were from 2.5 mg/ml to 10 mg/ml. The Results from this study validate the traditional use of *S. singueana* leaves in microbial infections. Further study on EE extract to identify the bioactive constituents responsible for this activity is recommended.

Keywords: Antimicrobial, *Senna singueana*, Phytochemicals, Minimum inhibitory concentration (MIC), Minimum Bactericidal concentration (MBC)

INTRODUCTION

Medicinal plants, rich in bioactive components, are gaining popularity due to their antimicrobial and antioxidant properties, with a global market worth $62 billion and increasing demand (Dhawan and Gupta, 2016, Talib & Mahasneh, 2010). Antimicrobial-resistant infectious diseases pose a significant public health threat, with the rapid global emergence of these bacteria threatening the effectiveness of prescribed antibiotics (Zeise *et al*., 2021, Van & Doi, 2017). This has been attributed to abuse of these medications and a lack of new drug development (Ousenu *et al.,* 2021). Medicinal plants overcome synthetic drug challenges like adverse effects, AMR, and low efficacy (Ripanda, 2023). Herbs gain attention as alternative medicines for infectious diseases but lack scientific evaluation and documentation.

Senna singueana (Delile) Lock, a tropical plant native to the Caesalpiniaceae family, can grow up to 15 meters. (Hiben *et a*l,2016). *S. singueana* is commonly called scrambled eggs, winter cassia, and sticky pods. Different parts of this plant have different medicinal uses all over Africa (Jambwa *et al.*, 2022). The leaves of the plant are traditionally used to treat fever, stomach cramps, conjunctivitis, and constipation, and are potential medicinal plants for various diseases. (Hiben,2016; Sobeh,2017; Jambwa *et al.,* 2022).

Studies suggest intestinal microbiota changes may contribute to constipation and related symptoms, with certain *E. coli, staph*, and fungi strains linked to urinary tract, stomach, fever, cramps, and pains. (Bein *et al*., 2012, Zeise *et al*.,2021). The study aims to validate the traditional use of *S.singueana* leaves, also known as Rumfu in northern Nigeria, for managing microbial infections.

MATERIALS AND METHODS

Plant Sample Collection, Authentication and Preparation

The fresh plant material (the leaves, fruits, and flowers) was collected in Samaru -Zaria. This was identified as *Senna singueana* at the herbarium section of the Department of Botany, Ahmadu Bello University, Zaria Nigeria, with voucher specimen number 6863. A sufficient quantity of the leaves was collected, dried at room temperature, powdered, and stored in a closed container for further use.

Extraction of the Leaves Powder

The plant material was extracted successfully in soxhlet apparatus as described by Kokate *et al,* (2006). Briefly, 600 g of pulverized plant sample was extracted using n-hexane, ethyl acetate, and methanol, concentrated, and stored in a desiccator for future use.

Preliminary Phytochemical Screening

Preliminary phytochemical tests for secondary metabolites such as triterpenes, flavonoids, cardiac glycosides, alkaloids, tannins, and saponins, were carried out according to standard procedures as described by Evans,2009 and Sofowora,2008.

Collection of Clinical Isolates and Preparation af Inoculum

Twelve (12) clinical isolates of fungi *(Candida krusei, Candida tropicalis, Candidia albican)*, **gram-positive stains** (*Staphylococcus aureus, Streptococcus faecalis)* and **gram-negative stains**; (*Campylobacter jejuni Escherichia coli, Neisseria gonorrhea Pseudomonas aeruginosa, Salmonella typhi, Shigella dysentery,*) were collected from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika – Zaria. Bacterial isolates were prepared in Muller Hinton Broth overnight, adjusted to 108 cfu/ml, and fungi subcultured in Saouraud dextrose broth, with Ciprofloxacin and fluconazole as positive control drugs.

Determination of Antimicrobial Activity of leaves of *S. singueana*

The agar well diffusion method was used for the antimicrobial screening. Solutions of the leaf extracts (hexane, ethyl acetate, and methanol extract) of varying concentrations were prepared. The media, Meuller Hinton agar (MHA) for bacteria and MHA supplemented with 2% glucose and 0.5% ug/ml methylene blue for the fungi species were sterilized at 121^{o} C for 15 min. The sterilized media were evenly seeded with the standard microbe's inoculum, using a sterile swab, and allowed to dry at room temperature. A 6mm Min diameter cork-borer was used to cut wells in inoculated mediums, and 0.1 ml of extract was introduced into each well. The plates were thereafter incubated at 37ºC for 24 hours and the plates were observed for zones of inhibition. The initial concentration was obtained by dissolving 0.2 g of the extract in 10 ml dimethyl sulfoxide (DMSO) to obtain a concentration of 20 mg/ml. This concentration was diluted to obtain 10mg/ml, 5mg/ml, 2,5mg/ml, 1.25mg/ml, and 0.63mg/ml respectively. (Gonfa *et al*., 2023, Adeyanju *et al*., 2012). DMSO was used as the negative control.

Minimum Inhibition Concentration (MIC) of Extracts

From the prepared 20mg/ml of extracts, a two-fold serial dilution of the extract was made to obtain the concentrations of 10mg/ml, 5 mg/ml, 1.25 mg/ml and 0.63 mg/ml. 1ml of the prepared inoculum was dispensed into sterile dishes and Mueller Hinton molten agar (10 ml) was added and mixed. Four wells were made on each plate and 0.1 ml of the different concentrations of each extract was transferred to the respective well. Plates were incubated at 37ºC for 24 hours. The lowest concentration of the extract in the plates which showed no growth was recorded as the MIC of each extract (Faraja *et al,* 2018).

Minimum Bactericidal and Fungicidal Concentrations (MBC/ MFC)

Mueller Hinton agar was prepared, sterilized at 121ºC for 15 minutes, poured into sterile Petri dishes, and allowed to cool and solidify. The content of the test tubes (plant extract) from the MIC that showed no growth was seeded on the prepared medium. Incubation was made at 37 ºC for 24 hours. The plate with the lowest concentration of extract without colony growth was the Minimum bactericidal / Fungicidal concentrations (MBC/MFC) of the extracts

RESULTS

Extraction of *S. Singueana* leaves.

Figure 1 showed that there was a significant difference in the extraction yield using different solvents (n-hexane, ethyl acetate and methanol). Methanol resulted in the highest extraction yield (14.32%), followed by ethyl acetate (7.37%) then n-hexane (2.18%)

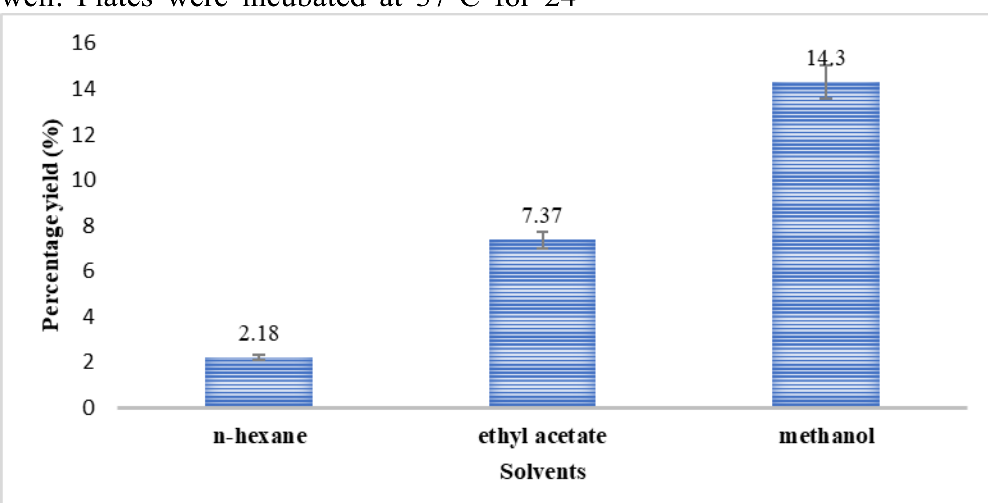

Fig. 1: Percentage yield from different solvents used in extraction

Preliminary Phytochemical Constituents

All the phytochemicals tested (alkaloids, triterpenes, saponins, tannins, anthraquinones, flavonoids, and cardiac glycosides) were present in methanol extract. Alkaloids, triterpenes, and flavonoids were present in all three extracts (hexane, ethyl acetate and methanol extracts). (Table 1).

Antimicrobial activity of the extracts

S. singueana leaves extracts showed both antibacterial and antifungal activities. The ethyl acetate extract was the most active of the three extracts as shown by the zone of inhibition in Table II below. The standard drug Ciprofloxacin and the three extracts showed no activity towards *C. jejuni and N.gonorheae.*

Table I: Qualitative phytochemical screening

Test	N-hexane	Ethyl acetate	Methanol
Alkaloids	+	+	+
Triterpenes	+	+	+
Saponins	-	-	+
Tannins	-	-	+
Anthraquinones	-	-	+
Flavonoids	+	+	+
Cardiac glycosides	-	+	+

Table II: Zone of inhibition of *S. singueana* extracts (10 mg/ml)

Microorganisms	**Hexane (mm)**	**Ethyl acetate (mm)**	**Methanol (mm)**	**Ciprofloxacin. (mm)**	**Fluconazole. (mm)**
Staphylococcus aureus	18	22	20	32	0
Streptococcus faecalis	19	25	21	37	0
Escherichia coli	17	27	21	38	0
Neisseria gonorrhoea	0	0	0	0	0
Salmonella typhi	18	21	20	41	0
Pseudomonas aeruginosa	0	0	0	32	0
Campylobacter jejuni	0	0	0	0	0
Proteus mirabilis	0	0	0	0	0
Shigella dysentery	0	0	0	40	0
Candida albicans	18	23	20	0	32
Candida krusei	0	0	0	0	37
Candida tropicalis	17	27	22	0	34

Minimum inhibitory concentration (MIC) of extracts against the microorganisms

Ethyl acetate showed the lowest concentration needed to inhibit the activity of the microbes, followed by methanol extract then, hexane extract. The MIC for *E. coli* was 1.25mg/ml, 2.5mg/ml, and 5mg/ml using ethyl acetate, methanol and hexane extract respectively (Table III).

Minimum Bactericidal Concentration (MBC) of extracts against the microorganisms

Ethyl acetate extract had the lowest MBC value of 2.5mg/ml against E. *coli* while the MIC value for hexane and methanol extracts was at 10mg/ml concentration against the tested organisms. This is shown in table IV below

Table III: MIC of *Senna singueana* extracts against the clinical isolates

	Hexane (mg/ml)					Ethyl acetate (mg/ml)					Methanol (mg/ml)				
Organisms	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63
S. aureus	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
S. faecalis	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
E. coli	-	*	+	++	+++	-	-	-	*	+	-	-	*	+	++
S. typhi	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
C.tropicalis	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
C. albican	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++

Keys: - no growth, * MIC, + (light growth), ++ (moderate growth), +++ (heavy growth

Table IV: Minimum bactericidal/fungicidal concentration of *S. singueana* extracts against the microbes (MBC/MFC)

Organisms	Hexane (mg/ml)					Ethyl acetate (mg/ml)				Methanol (mg/ml)					
	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63
S. aureus	*	+	++	+++	+++	*	+	++	+++	+++	*	+	++	+++	+++
S. faecalis	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
E. coli	*	+	++	+++	+++	-	-	*	+	++	*	+	++	+++	+++
S. typhi	*	+	++	+++	+++	*	+	++	+++	+++	*	+	++	+++	+++
C. tropicalis	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
C. albicans		+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++

Keys > (*) = MBC/MFC, (-) No colony growth, (+) scanty growth, (++) moderate growth, (+++) heavy growth.

DISCUSSION

To obtain bioactive compounds from plants, extraction is one of the several important steps to be taken. The efficiency of extraction is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals, and the solvent used (Turkmen *et al.,*2006, Simorangkir *et al*., 2019). In this present study, *S. singueana* dried powdered leaf was extracted successfully using n-hexane, ethyl acetate, and methanol in a Soxhlet apparatus. Different solvents (n-hexane, ethyl acetate, and methanol) were used for the extraction to study the effects of solvents types on the extraction yield. A higher extraction yield was observed in methanol extract followed by ethyl acetate then hexane. This result is consistence with the extraction yield of *Senna alata* (Owoyale *et al*., 2006), and some other medicinal plants (Gonzfa *et al*. (2023), Dhawan & Gupta (2016), Kolawole *et. al, (*2021), Ripanda, (2023*)*. The high yield of methanol extract is attributed to its high polarity. It also means the plant material contains a higher number of polar compounds soluble in high polar solvents. This was observed from the result of the preliminary phytochemical study (Table 1) where Methanol indicated a positive result for all phytochemicals tested in this study. Alkaloids, flavonoids, and triterpenes were present in the three extracts. flavonoids and alkaloids have been found to possess antibacterial activity in various species. (Shamsudin *et al.,* 2022, Mishara *et.al*., 2013; Cushnie and Lamb 2011; Ebel *et al.,* 2004). Alkaloids, with their bitter taste, are toxic to other organisms, inhibiting microorganism growth through mechanisms such as nucleic acid and protein synthesis. (Yan *et al.,* 2021, Enwa *et al*., 2014; Gupta *et al.,* 2010). Indoquinoline alkaloids have been found to be effective against gram-negative bacteria such as *E. coli. and S. typhi*. (Vaou *et al*., 2021*,* Silva *et al*, 1996)

This study also evaluated the antimicrobial activity of three extracts (hexane, ethyl acetate and methanol extracts). A wide zone of inhibition against tested microbes was observed. Ethyl acetate extract was observed with the highest zone of inhibition ranging from 23mm to 27mm among the three extracts. Several studies have shown that the wider the zone of inhibition, the lower the concentration of antimicrobials needed to inhibit organism growth, And hence, the higher the activity (Ripanda, 2023; Yan *et al.,* 2021; Adeyanju *et al*., 2011). This indicates that ethyl acetate extract of *S. singueana* leaves is the most active of the three extracts used in this study.

In Table II, the three extracts were reactive to some of the organisms as indicated by their zones of inhibition to both bacterial, and fungi organisms, such as *E. coli, S. typhi, S. aureus, S. faecalis, and C.albican and C. tropicalis,* however, some of the tested microorganisms (*S. dysentery, N. gonorrhoea, C. jejuni, P. mirabilis, P. aeruginosa, and C. krusei)* were found to be resistant to all the extracts. The three fundamental reasons for antimicrobial resistance are; enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics (Rodríguez-Melcón *et al*.,2021, Enwa *et al.*,2014, Cushnie and Lamb, 2011). *N. gonorrhoea, C. jejuni, and C. krusei* were also resistant to the standard drugs Ciprofloxacin and fluconazole respectively. Several studies including (Rahman *et al*., 2001, Mishra *et al*.,2022) have reported resistance cases of antibiotics such as ciprofloxacin in the treatment of gonorrhoea and other infections.

The plant extracts showed activities against the tested organisms. Ethyl acetate extract showed the lowest concentrations and a positive correlation between its MIC values (1.25 mg/ml to 5 mg/ml) and MBC/MFC values (2.5 mg/ml to 10 mg/ml) (Tables III and IV). The MIC and MBC/MFC values are crucial for determining effective doses of antimicrobials, with lower values indicating higher potency. (Mostafa *et al*., 2018). Using ethyl acetate extract, a lower concentration of extract is needed to inhibit or kill the microorganisms. The MIC and MBC/MFC values for *E. coli* and *C. albican* were 1.25 mg/ml and 2.5 mg/ml, respectively.

Ethnomedicinally, *S. singueana* leaves extract is used to manage microbial infections such as UTIs, gastroenteritis, stomach pains, etc. Bacterial UTIs in hospitalized patients are primarily caused by *E. coli (50% cases), S. aureus (10% cases)*, and other gram-negative bacteria, with *C. albican* being the most common fungi isolate. (Mishra *et.al*.,2022, Imam, 2023). Also, *E coli* and *S.typhi* are commonly implicated in gastroenteritis (Ousenu *et* *al.,*2021. Hence, the use of the plant *S. singueana* in the management of microbial infections ethnomedicinally is justified.

CONCLUSION

The study reveals that *S.singueana* leaves extracts possess antimicrobial properties, supporting their ethnomedicinal uses in managing microbial infections like urinary tracts infections (UTIs) and gastroenteritis. With ethyl acetate extract being the most active, further research is needed to identify the phytochemicals and the mechanism of action responsible for this antimicrobial activity in *S. singueana* ethyl acetate leaves extract.

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