

Comparative study of in-vitro antimicrobial activity and phytochemical composition of *Sida cuneifolia* fruits, leaves, and stem bark extracts

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ABSTRACT

Background: *Sida cuneifolia* plant parts are important in ethno-veterinary medicine, but no studies exist on a comparative evaluation of phytochemical composition and antimicrobial properties of fruits, stem bark and leaves. This study evaluated and compared extraction yields, phytochemical composition as well as antibacterial and antifungal properties of ether, methanol and aqueous crude extracts of fruits, leaves, and stem bark of *S. cuneifolia*.

Methods: Yields of extracts were determined, and qualitative phytochemical screening tests were carried out to establish their composition. Crude extracts were screened against five bacteria and two fungi. Agar disk diffusion technique was used to determine the inhibition zone diameters. Broth micro-dilution method was used to determine the minimum inhibition concentrations (MIC) and minimum bactericidal concentrations (MBC).

Results: Triterpene aglycones, sterols, tannins, atheracene derivatives, polyuronides and glucides were found in intense mounts in *S. cuneifolia* fruits. Leaves contained intense amounts of carotenoids, tannins, polyuronides and coumarin derivatives. For the leaves, methanol extracts had MICs and MBCs as low as (≤ 3.13 mg/ml) against the majority (6/7) microorganisms hence had the best activity. Considering the stem bark, the ether extracts had the best antimicrobial activity with MICs as low as ≤ 3.13 mg/ml against 6/7 microorganisms. For the fruits, the ether extracts had the best antibacterial activity with MICs and MBCs as low as (≤ 3.13 mg/ml) against all tested bacteria.

Conclusion: The study provides scientific evidence for ethno-veterinary use of *S. cuneifolia* leaves, fruits and stem bark, and this can be exploited in the transformative development of ethno-medicine.

Keywords: Antibacterial, Antifungal, Phytochemistry, Poultry, *Sida cuneifolia*

INTRODUCTION

Sida cuneifolia is a small shrubby herb with yellow mallow flowers, five distinct petals and five sepals. The leaves are notched at the tip, and the fruit consists of five mericarps.¹ *S. cuneifolia* belongs to family malvaceae and genus *Sida*, which has about 200 species distributed in the tropical and sub-tropical regions of the world. Species of genus *Sida* are medicinal plants of great value because of their use in the preparation of indigenous drugs in Asia and Africa.² Various *Sida* species such as *Sida acuta*, *Sida spinosa*, *Sida carpenifolia*, *Sida humilis*, and *Sida veronicaefolia* are used in Ayurvedic system of medicine and in some African herbal

treatment.²⁻⁴ *S. cuneifolia* has been used in folk medicine in Uganda and other parts of Africa.⁵⁻⁹ As part of its uses, *S. cuneifolia* plant is used in the management of poultry diseases.¹⁰ Given the importance of *S. cuneifolia* there is a need for research to validate its use. Although some studies exist on antibacterial activity of leaves and roots of *S. cuneifolia*,^{6,11} there are no reports on the antifungal activities of leaves. In addition, no studies have been reported on phytochemical composition and efficacies of extracts from fruits and stem barks of *S. cuneifolia*. This study, therefore, aimed at evaluating and comparing the antimicrobial activity of the leaves, fruits and the stem bark of *S. cuneifolia* against selected bacterial and fungal poultry disease causing

pathogens; and the phytochemical composition of these plant parts. Findings from this study will contribute to information that will form the basis for promotion of *S. cuneifolia* use in poultry disease management.

METHODS

Plant collection and identification

The plant was collected from Mukungwe sub-county, Masaka district in the central Uganda. The plant voucher specimen was prepared, identified and preserved as *S. cuneifolia* Roxb. (RN02) at the Botany unit herbarium of Makerere University. Ethical clearance was obtained from National Council of Science and Technology, reference number, HS 1561.

Preparation of plant extracts

The leaves, stem bark and fruits were excised from *S. cuneifolia* whole plant. The leaves and stem barks were collected before the flowering and fruiting period. These plant parts were cleaned and air-dried under a shade at room temperature (24°C) for 2 weeks. The dried samples were pulverized using an electrical grinder and sieved to obtain fine powder. The solvents used were distilled water (aqueous), methanol and diethyl ether. The aqueous extracts were prepared by boiling the powder in water for 25 mins and later filtered using cotton wool to remove coarse particles and then through a filter paper (Whatman No.1, England) in Buchner funnel to get a clear filtrate. After extraction, the aqueous extract was freeze-dried. The methanol and ether extracts were prepared using cold maceration methods. Briefly, the grounded powder were weighed and soaked in the solvents for 3 days at room temperature, 24°C with occasional shaking. The mixtures were filtered through cotton wool and later through filter paper (Whatman No.1) in Buchner funnel to get a fine filtrate. The methanol and ether extracts were concentrated by evaporation using a rotarvapor (Perkins, UK) under reduced pressure at 40°C, weighed and stored.

From the above procedures, aqueous, methanol and ether crude extracts were obtained. The percentage extraction yield was calculated using the formula:¹²

$$\text{Percentage of extraction (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

The crude extracts were then kept in a refrigerator at 4°C until use for proceeding tests.

Phytochemical composition screening tests

The extracts were screened for presence of phytochemical constituents using standard methods as reported by various researchers. To test for the presence of alkaloids, Mayer's reagent was used while Borntrager's test tested for the

presence of anthracenes.¹³ Carr-Price's reaction detected presence of carotenoids while volatile oils were confirmed by steam distillation.¹⁴ The presence of sterols, triterpenes and cardiac glycosides was detected by Liebermann-Burchard's test and Kellar-Killiani test, respectively.^{13,15} Whereas the foam test detected presence of saponins.^{13,16,17} Presence of reducing sugars was detected by Fehling's test.¹⁸ While glucides were detected by Molisch's reagent.^{13,19} Shibata's reaction was used to confirm presence of flavonoids.¹⁶ Tannins were detected using Braemer's test¹⁵ and to differentiate between the tannins, when three drops of ferric chloride were added to 1 ml of methanol and of the aqueous extract, a black to red color indicated gallic tannins, while green to black color was evidence of catechol tannins.²⁰ Coumarins were tested by evaporating ether extract to dryness and dissolving the residue by heating in 2 ml of water and they were confirmed if 0.5 ml of 10% ammonia solution was added and a blue or green fluorescence under ultraviolet light developed. Presence of anthocyanin pigments was detected if the methanol and aqueous extract turned pink or red in an acidic medium and green or blue in an alkaline medium.²¹ To detect for the presence of polyuronides, about 2 ml of aqueous extract were added dropwise to 10 ml of ethanol. Formation of a thick precipitate indicated presence of polyuronides.¹⁶ The formed precipitate was separated off and washed away with ethanol and stained with methylene blue and occurrence of violet color further denoted presence of polyuronides.

Establishment of antimicrobial activity

Preparation of the microbial inoculum

Five bacterial species were used for antibacterial screening tests. These included reference cultures, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922); and wild type cultures *Salmonella gallinarum*, *Salmonella typhimurium* and *Pasteurella multocida* obtained from the Veterinary Microbiology Laboratory at Makerere University. These bacterial species are responsible for a cross-section of poultry disease conditions namely; staphylococcosis, colibacillosis, fowl typhoid, paratyphoid and fowl cholera, respectively. Two wild type fungal species obtained from the Veterinary Microbiology Laboratory of Makerere University were also used for antifungal screening tests. These included *Aspergillus flavus* and *Candida albicans*, which are responsible for pneumomycosis and candidiasis, respectively.

The inocula were prepared by culturing each of the bacterial strains on Mueller Hinton agar (Zayo-Sigma, Germany) for 24 hrs at 37°C. Then, a standardized bacterial suspension was prepared by picking a colony of respective bacteria and suspending it in 5 ml of brain heart infusion broth (Mast Diagnostics, UK). The inoculated broth was vortexed, and turbidity adjusted to 0.5 McFarland standard (ca. 1.0×10^8 CFU/ml). For fungi, the inocula, in the form of spore suspensions were prepared by diluting

in sterile potato dextrose broth (PDB)(Oxoid, Spain) and standardized to 0.5 McFarland standard (ca. 10^7 spores/ml). The McFarland standard was prepared by adding 0.5 ml of 0.048 M BaCl_2 (1.17% w/v $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) to 99.5 ml of 0.18M H_2SO_4 (1% v/v) with constant stirring.²² The standard was vigorously agitated on a vortex mixer before use. After comparison with McFarland standard, the final dilutions formed the bacterial and fungal inocula for use in the bioassays as outlined below.

Screening for antimicrobial activity

Disc diffusion method was used to determine the inhibition zone diameters.^{23,24} Briefly, media included Mueller Hinton agar (Zayo-Sigma, Germany) and potato dextrose agar (Oxoid, Spain) for bacterial and fungal growth-inhibition analyses respectively. Dry, plant extracts were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 50 mg/ml, which was used for the tests.

For antibacterial assays, the standardized bacterial suspension was surface spread on Mueller Hinton agar; while potato dextrose agar was used for antifungal assays, where the standardized spore suspension was surface spread using a sterile cotton swab. The inoculated plates were covered and allowed to dry for 4 mins. Three sterile Whatman filter paper No. 1 discs of 6 mm diameter were placed equidistantly on each of these plates. The filter paper discs were then impregnated with 10 μL of each plant extract of concentration of 50 mg/ml. Negative control discs were impregnated with 10 μL DMSO. Standard antibacterial discs of gentamicin (10 μg) and ciprofloxacin (5 μg) served as positive controls; while for fungi, Nystatin (100 IU) discs were used. All tests were run in triplicates to ensure reliability. The plates were then incubated at 37°C for 24 hrs and at 28°C for 48 hrs for bacteria and fungi, respectively; after which they were examined for growth-inhibition zone diameters. The inhibition zone diameter was measured, and the mean, and standard errors of three replicates were recorded. Results were presented using activity scale according to Monks et al.²⁵ who categorized inhibition zones as no activity (<7 mm); weak activity (7-11 mm); moderate activity (11-16 mm); high activity (>16 mm).

Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

Using broth micro-dilution method, MICs for the extracts were determined against bacteria and fungi and MBCs were additionally determined against bacteria.^{23,24} For bacteria, 50 μL of Mueller Hinton broth (Himedia, India) was placed into each well of a 96-well micro-plate and iso-volumes of the extracts (12.5 mg/ml) were added to the first well and serially diluted (two-fold) 8 times and the final dilution was 0.098 mg/ml. Each type of extracts was tested in triplicates. All the wells were then filled with 50 μL of the test bacterial

inoculum. The antibiotics, gentamicin and ciprofloxacin, were included as positive controls in each assay. A 100% DMSO solution was used as a negative control. The micro-plates were incubated overnight at 37°C. The MICs were recorded as the lowest concentration of the extract that completely inhibited bacterial growth as observed by the naked eye. From all the wells which did not show any turbidity, the suspension was streaked on Mueller-Hinton agar plates. The lowest concentrations of extract that did not permit any visible growth on the plates after 48 hrs of incubation at 37°C were recorded as MBCs.

For fungal inhibition studies, the procedure was as described for the determination of MICs against bacteria, except, PDB was the medium for growth and Nystatin was used as a positive control in each assay. In addition, the micro-plates were incubated at 28°C for 48 hrs in case of *C. albicans* and 7 days for *A. flavus*. The MICs were recorded as the lowest concentration of the extract that completely inhibited fungal growth by the naked eye.

RESULTS

Extraction yields

As shown in Figure 1, the percentage extraction yield ranged from 1.5% to 44.8%. The aqueous extracts showed the maximum percentage yields of 12.1%, 19.5%, and 44.8% from fruits, leaves, and stem bark respectively. Compared with the leaves and fruits, the stem bark produced the highest yield by the different solvents, water yielding (44.8%), methanol (9.5%) and diethylether (3.1%).

Phytochemical composition of *S. cuneifolia*

The preliminary phytochemical study revealed that the extracts from the fruits, leaves and stem bark of *S. cuneifolia* had a variety of phytochemicals (Tables 1 and 2). Out of

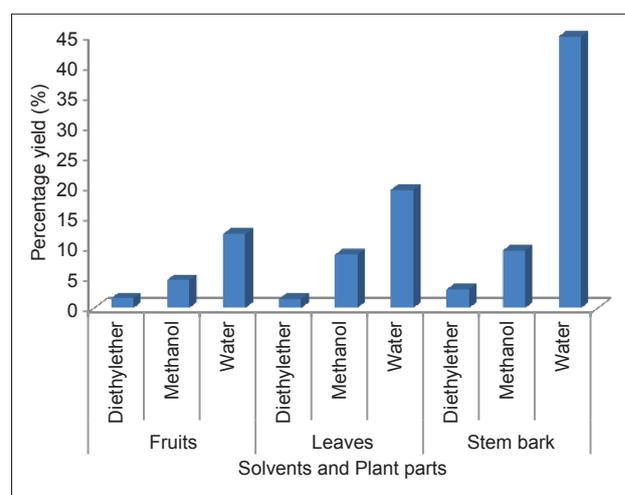


Figure 1: Percentage extraction yield of extracts from the fruits, leaves and stem back of *Sida cuneifolia*.

the 20 phytochemicals screened, the leaves and the fruits exhibited 19, while the stem bark exhibited 15.

The diethyl ether extracts from the seeds, leaves and stem bark of *S. cuneifolia* had a number of non-polar phytochemicals (Table 1). The leaves exhibited all the eight non-polar phytochemicals while fruits and stem bark exhibited seven and six respectively. The triterpene aglycones and sterols were present in fruits in high intensity (+++) while carotenoids were highly intense (+++) in fruits.

Table 2 presents the polar phytochemicals encountered in the methanol and aqueous extracts of the fruits, leaves and stem bark of *S. cuneifolia*. Fruits contained all the 12 polar phytochemicals examined, while leaves and stem bark

contained 11 and nine, respectively. Tannins, polyuronides, athracene derivatives, and glucides were highly concentrated (+++) in fruits while leaves contained a high intensity (+++) of tannins, polyuronides and coumarins.

Antimicrobial activity of *S. cuneifolia* fruits, leaves and stem bark extracts

The results of the in-vitro antibacterial and antifungal activity of the aqueous, methanol and ether extracts from the fruits, leaves and stem bark of *S. cuneifolia* determined by the inhibitory zone diameters (in mm) are presented in Table 3. Among all the extracts from the leaves, fruits, and stem bark of *S. cuneifolia*, methanol extracts were active against most of the tested organisms. Methanol leaf and fruit extracts were active against all the seven tested microorganisms while methanol stem bark extracts were active against six of them. Methanol extracts most of the time exhibited moderate (11-16 mm) and high (>16 mm) activities, followed by ether and then aqueous extracts. All the extracts showed activity against *P. multocida* and inhibition zones with leaf and fruit methanol and ether extracts exhibited high activity comparable to the standard synthetic antibacterials.

Table 4 presents the MICs and the MBCs (in mg/ml) of the active aqueous, methanol and ether extracts from the leaves, fruits and stem bark of *S. cuneifolia*. Considering the leaves, the methanol leaf extracts had the lowest MICs and MBCs (≤ 3.13 mg/ml) against the majority (6/7) of the tested microorganisms. For the fruits, the ether fruit extracts had the lowest MICs and MBCs (≤ 3.13 mg/ml) against all tested bacteria. As for the stem bark, the ether stem bark extracts had the lowest MICs (3.13 mg/ml) against 6/7 microorganisms, but demonstrated high MBCs (> 3.13 mg/ml). All the aqueous extracts had high

Table 1: Phytochemicals present in the diethyl ether extracts in the fruits, leaves and stem bark of *S. cuneifolia*.

Plant constituents	Diethyl ether extracts		
	Fruits	Leaves	Stem bark
Anthracene aglycones	-	+	+
Flavone aglycones	++	++	+
Coumarins	+	+	-
Basic alkaloids	+	+	++
Carotenoids	++	+++	+
Triterpene aglycones	+++	+	+
Sterols	+++	+	+
Volatile oils	++	+	-

+++ : Presence of phytochemical with very intense colour change, ++ : Presence of phytochemical with definite color change, + : Presence of traces of phytochemical with faint colour change, - : Absence of phytochemical, *S. cuneifolia*: *Sida cuneifolia*

Table 2: Phytochemicals present in methanol and aqueous extracts in the fruits, leaves and stem bark of *S. cuneifolia*.

Phytochemical constituents	Fruits		Leaves		Stem bark	
	ME	AE	ME	AE	ME	AE
Saponins	++	-	+	+	+	++
Tannins	+++ (g)	++ (c)	+++ (g)	+++ (g)	++ (g)	++ (g)
Akaloid salts	++	+	+	-	+	+
Athracene derivatives	+++	+	++	++	+	+
Coumarin derivatives	+	++	+	+++	+	++
Steroid glycosides	++	-	+	-	-	++
Flavone glycosides	+(r)	+(r)	+(r)	+(r)	++(r)	+(r)
Anthocyanin pigments	+	++	+	+	+	+
Reducing compounds	+	ND	++	ND	-	ND
Poly uronides	ND	+++	ND	+++	ND	+
Glucides	ND	+++	ND	++	-	-
Cardiac glycosides	++	++	-	-	-	-

+++ : Presence of phytochemical with very intense colour change, ++ : Presence of phytochemical with definite color change, + : Presence of traces of phytochemical with faint colour change, - : Absence of phytochemical, ME: Methanol extract, AE: Aqueous extract, g: Gallic tannins, c: Catechol tannins, r: Flavonols, ND: Not done, *S. cuneifolia*: *Sida cuneifolia*

MICs and MBCs of ≥ 12.50 mg/ml and were active against *P. multocida* only.

DISCUSSION

Some plants contain chemical components that are biologically active and therefore have various parts such as; leaves, roots, rhizomes, stems barks, flowers, fruits, grains or seeds, employed in the control or treatment of various disease conditions.²⁶ In this study, the antibacterial and antifungal properties; as well as phytochemical composition of the fruits, leaves and stem barks of *S. cuneifolia* were evaluated and compared.

The three solvents, diethyl ether, methanol and water exhibit different polarities and were used to obtain different amounts of extract from the fruits, leaves and stem bark of *S. cuneifolia*. Water extracted the highest amounts of extract from the fruits, the leaves and the stem bark; while methanol had moderate extraction yields. In case aqueous extracts are to be utilized for any scientific studies, the potential of water to extract high yield lessens plant destruction and promotes protection that makes it advantageous. Besides, water is a cheap and ubiquitous solvent and it's what is used mostly by the local people in processing of ethnomedicinal preparations.

Table 3: The antibacterial and antifungal activity (inhibition zone diameters given in mm) of three *S. cuneifolia* plant parts against seven pathogens.

Plant part and extract	Bacteria species tested					Fungal species tested		
	Sa	Ec	Sg	St	Pm	Af	Ca	
Leaf								
Aqueous	-	-	-	-	**	-	-	
Methanol	*	*	**	**	***	*	*	
Ether	**	**	-	-	***	-	-	
Fruit								
Aqueous	-	-	-	-	**	-	-	
Methanol	*	**	*	*	**	*	*	
Ether	*	*	*	*	***	-	-	
Stem bark								
Aqueous	-	-	-	-	**	-	-	
Methanol	**	**	*	*	***	-	**	
Ether	***	*	*	*	***	-	**	
Positive controls								
Ciprofloxacin	***	***	***	***	***	NA	NA	
Gentamycin	***	***	***	***	***	NA	NA	
Nystatin	NA	NA	NA	NA	NA	**	***	

Sa: *Staphylococcus aureus* (ATCC 25923), Ec: *Escherichia coli* (ATCC 25922), Sg: *Salmonella gallinarum*, Ca: *Candida albicans*, NA: Not applicable, *** >16 mm (high activity), **11-16 mm (moderate activity), *7-11 mm (weak activity), <7 mm (No activity), *S. cuneifolia*: *Sida cuneifolia*

The qualitative phytochemical analysis of extracts of fruits and leaves had substantial quantities of some phytochemicals. This was true for triterpene aglycones and sterols in ether fruit extracts, tannins and athracene derivatives in methanol

Table 4: The MICs and MBCs for *S. cuneifolia* fruits, leaves and stem bark extracts against seven pathogens.

Microbe	MIC/MBC (mg/ml)		
	Fruit	Leaf	Stem bark
Methanol extract			
<i>S. aureus</i>	3.13/6.25	0.39/0.78	1.56/3.13
<i>E. coli</i>	3.13/6.25	0.39/0.78	1.56/3.13
<i>S. gallinarum</i>	6.25/12.50	0.78/1.56	6.25/12.50
<i>S. typhimurium</i>	6.25/12.50	0.78/1.56	3.14/6.25
<i>P. multocida</i>	6.25/12.50	0.39/0.39	6.25/12.50
<i>C. albicans</i>	≥ 12.50	3.14	6.25
<i>A. flavus</i>	≥ 12.50	12.50	ND
Ether extract			
<i>S. aureus</i>	1.56/3.13	6.25/12.50	3.13/12.50
<i>E. coli</i>	1.56/3.13	0.20/0.39	0.78/1.56
<i>S. gallinarum</i>	1.56/3.13	ND	3.13/6.25
<i>S. typhimurium</i>	1.56/3.13	ND	3.13/6.25
<i>P. multocida</i>	0.78/1.56	0.39/0.78	0.39/0.78
<i>C. albicans</i>	ND	ND	1.56
<i>A. flavus</i>	ND	ND	ND

ND: Not done, *S. cuneifolia*: *Sida cuneifolia*, MIC: Minimum inhibition concentrations, MBC: Minimum bactericidal concentrations, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *S. gallinarum*: *Salmonella gallinarum*, *S. typhimurium*: *Salmonella typhimurium*, *P. multocida*: *Pasteurella multocida*, *C. albicans*: *Candida albicans*, *A. flavus*: *Aspergillus flavus*

Table 5: Comparing the current study and previous study on the phytochemical composition of *S. cuneifolia* leaves.

Phytochemical constituents	CS	PS
Saponins, tannins, alkaloid salts and basic alkaloids, coumarin derivatives and coumarins, steroid glycosides, sterols and triterpene aglycones, flavone glycosides and flavone aglycones, poly uronides/mucilages, carbohydrates/glucides	Present	Present
Reducing compounds, carotenoids	Present	Absent
Athracene derivatives and athracene aglycones, anthocyanin pigments, volatile oils	Present	ND
Cardiac glycosides	Absent	ND

CS: Current study on phytochemical composition of *S. cuneifolia* leaves; PS: Previous study done on phytochemical composition of *S. cuneifolia* leaves.¹¹ ND: Not done. *S. cuneifolia*: *Sida cuneifolia*

fruit extracts as well as polyuronides in water fruit extracts. The same scenario was also observed for carotenoids in ether leaf extracts, tannins in the water and methanol extracts as well as polyuronides and coumarin derivatives in water leaf extracts. The existence of phytochemicals in appreciable amounts makes these plant parts reliable sources that can be advantageous if they are to be utilized for any biological activities. The rest of the phytochemicals present existed in either moderate or trace amounts and to the best of our knowledge, these have been reported for the first time in the fruits and stem bark of *S. cuneifolia*. In the case of leaves, reports¹¹ on phytochemical composition exist but several phytochemicals were either not tested for or reported to be completely absent as presented in Table 5. The probable reason for the disparity in the results for the two studies can be due to the differences in the sources of samples as well as variation of harvesting periods. In addition, the current study used methanol as one of the solvents while the previous study by Nalubega et al.¹¹ used ethanol and much as both are alcohols, methanol extracts give wider varieties of phytochemicals.

Different phytochemicals that could probably have contributed to antimicrobial activity were present in the fruits, leaves and stem bark of the different extracts. Basic alkaloids and alkaloid salts were present in the fruits, leaves and stem bark of *S. cuneifolia* and earlier studies have demonstrated that alkaloids exhibit good antimicrobial activity.^{13,27,28} Flavone aglycones and flavones glycosides were present in the fruits, leaves and stem bark of *S. cuneifolia* and as reported in previous studies, flavonoids also exhibit antimicrobial activity.²⁹ However, this could be more applicable for flavonoids found in methanol extracts since flavonoids in the aqueous extracts generally possess poor antimicrobial activity as acknowledged in earlier studies.¹³ This can probably explain the very poor activity exhibited by all the aqueous extracts from the different plant parts. Tannins were present in high intensity in fruits and leaves and moderate intensity in the stem bark of *S. cuneifolia* and the fact that these exhibit antimicrobial activity has been acknowledged by several studies.³⁰⁻³² Triterpene aglycones, sterols and steroid glycosides were present in the fruits, leaves and stem bark of *S. cuneifolia* while volatile oils were restricted to fruits and leaves. The terpenes and terpenoids such as the volatile oils the triterpenes and their derivatives are said to exhibit antimicrobial properties according to Dorman and Deans.³³ Saponins were present in the fruits, leaves and stem bark of *S. cuneifolia* and these have also been acknowledged to exhibit antimicrobial activity.^{34,35} It is likely that some of these phytochemicals contributed to antimicrobial activity of the active extracts.

Methanol extracts mainly exhibited moderate (11-16 mm) and high (>16 mm) antimicrobial activities exhibited when compared to ether and aqueous extracts and this was probably because methanol extracted a wider variety of active phytochemicals. In addition, alcohol solvents

such as methanol are very efficient in cell wall, and seed degradation hence are capable of extracting a variety of active phytochemicals.¹³ The antimicrobial activity exhibited by methanol leaf extracts against six of the seven microorganisms at low concentrations (≤ 3.13 mg/ml) further shows evidence of good activity by the methanol extracts. Unlike the ether leaf extracts and ether stem bark extracts, the ether fruit extract showed reasonably good antibacterial activity with low MICs and MBCs (≤ 3.13 mg/ml) signifying that non-polar phytochemicals with best antibacterial activity were in the fruits. The comparatively good activity demonstrated by ether fruit extracts relative to other fruit extracts further established their dependable activity. The good activity of ether fruit extracts can probably be partly explained by the high intensity of the triterpenes since they have been previously reported to exhibit good antimicrobial activity.³³ The study has shown that aqueous extracts exhibited poor activity since they had no activity against all the microbes, with the exception of *P. multocida*. This study differs from what was demonstrated in the previous study, which showed some activity of aqueous extracts against at least *S. aureus*.¹¹ This difference can however be explained by the high concentrations (1 g/ml) used for determination of inhibition zone diameters in the previous study as compared to the current study which used 50 mg/ml. A concentration of 50 mg/ml was used in the current study since low concentrations may not be toxic to animals and require less plant extract, which reduces plant destruction. The current study has further shown that, generally the fungi are less sensitive to all the extracts as compared to the bacteria. Though carried out on dichloromethane/methanol root extracts of *S. cuneifolia* plant, studies by van Vuuren and Viljoen⁶ showed the same trend where the fungi were less sensitive compared with the bacteria. This can, therefore, indicate that the different plant parts of *S. cuneifolia* are better to be utilized as antibacterials than as antifungals. To the best of our knowledge, this is the first study that established the antibacterial, antifungal and phytochemical properties of the fruits and stem bark of *S. cuneifolia*. In addition, this is the first study that has demonstrated the antifungal properties for the leaves of *S. cuneifolia*. Furthermore, the MICs and MBSs for the ether leaf and methanol leaf extracts of *S. cuneifolia* have not been reported in any other studies before.

In conclusion, *S. cuneifolia* plant parts exhibit a variety of phytochemicals that can be extracted with polar and non-polar solvents. In fruits, triterpene aglycones, sterols, tannins, atheracene derivatives, polyuronides and glucides exist in considerable amounts. Leaves also contain significant amounts of carotenoids, tannins, polyuronides and coumarin derivatives. The extracts are active on a number of microorganisms especially bacteria. In this study, we have only given the qualitative aspects of the phytochemicals. Further work should be done on the quantitative aspects and to establish the active compounds responsible for antimicrobial activity of the crude extracts.

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Conflict of interest: The researchers declare no conflicts of interest.

Ethical approval: Ethical clearance was obtained from National Council of Science and Technology, reference number, HS 1561.

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