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Full Length Research Paper

Preliminary phytochemical and antimicrobial screening of *Agave sisalana* Perrine juice (waste)

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Phytochemical and antimicrobial properties of the aqueous and methanolic extract of the *Agave sisalana* Perrine juice (waste) were investigated to evaluate its medicinal properties. The phytochemical screening reveals the presence of saponins, glycosides, phlobatannins, terpenoids, tannins, flavonoids, cardiac glycosides in the aqueous extract of the *A. sisalana* Perrine juice (waste). Steroids were absent. The methanol extract of sisal juice has more effect on *Shigella dysenteriae* and least on *Bacilus atrophaeus.* The study revealed that *A. sisalana* Perrine juice (waste) has the potential to be used for treatment against pathogenic organisms.

Key words: *Agave sisalana* Perrine, phytochemical, antimicrobial, medicinal properties.

INTRODUCTION

Plants offer a large range of natural compounds belonging to different molecular families which have various properties to humans (Hervé et al., 2008). *Agave sisalana*, popularly known as Sisal, belongs to the family Agavaceae and is a monocotyledonous plant, which grows mostly in countries like China, Brazil, Mexico, Tanzania, South Africa and Mozambique (Silva and Beltrão, 1999).

The search for natural products from agro-industrial waste, which may become useful to society, has grown in recent years. Sisal leaf decortication residue is one of the most abundant agro-industrial residues in Tanzania. Only 5% of the decortications of the leaves of Sisal (*A. sisalana*) produce the hard fiber that is used for various purposes; the remaining 95% consists of solidwaste (mucilage) and waste liquid (juice of the sisal) that are normally discarded by sisal farms (Oashi, 1999).

These wastes are untreated, disposed off and in most cases burnt, dumped in water bodies and/or landfilled; such practices are not sustainable and contribute to environmental pollution (Yu et al., 2002). Sisal waste principally contains plant tissues (ligninand cellulose), primary and secondary metabolites, and water. In an attempt to utilize this waste, some small local producers have systematically applied this residue to crops in an attempt to obtain improved production, or in the feeding of animals. Sisal waste has also been used as fertilizer (Lacerda et al., 2006), pesticides (Baker, 2003) and also animal feed (Faria et al., 2008). Pizarro et al. (1999) reported that the sisalwaste has insecticidal properties particularly against larvae of mosquitoes, which transmit tropical diseases. Plants are rich in secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties *in vitro*. Extracts of many plants are known to exhibit antimicrobial activity.

In this work, we evaluated the antimicrobial and phytochemical properties of *A. sisalana* juice *(*sisal waste) used against some clinical isolates.

MATERIALS AND METHODS

Extraction of extracts

The Sisal was harvested in Zaria, Kaduna State, Nigeria, within the premises of the National Research Institute for Chemical Technology, Zaria. The sisal juice was collected directly after mechanical decortication of the leaves in the laboratory. The juice was subjected to compression and the resultant liquid was filtered and dried under a controlled steam temperature (70°C) to obtain the crude extract of the sisal. 50 ml of the juice were cold-extracted using 50 ml of methanol and kept for five days at room temperature then filtered and dried under a controlled steam temperature

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(70°C), obtaining the methanol extract of the Sisal.

Test organisms

The standard strains of microorganisms (*Shigella dysenteriae, Bacillus atrophaeus, Enterococcus faecalis, Pseudomonas aeruginosa,* and *Candida albicans*) were obtained from Department of Medical Microbiology Laboratory of the Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria.

Phytochemical screening

Phytochemical analysis of the crude extract was performed according to standard method by Edeoga et al. (2005). The phytochemicals analyzed were flavonoids, saponins, tannins, phlobatannin, terpenoids, steroids, cardiac glycosides and glycosides.

Test for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowora, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for phlobatannins

There was deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous phlobatinins.

Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and about 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test)

5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer. Trease andEvans (1989).

Antimicrobial screening of extracts

The antimicrobial screening of the plant extracts was determined using agar well diffusion method as described by Irobi et al. (1994). The microorganisms were sub-cultured into prepared normal saline and incubated at 37°C for 30 min. The concentration of each suspension was obtained to form a turbidity that matched with scale 0.5 of Mac Farland's standard (1.5×10^8 cells/ml).

The cells suspensions of *E. faecalis* were *seeded* in Muller Hinton agar. *C. albicans*, *P. aeruginosa* and *B. atrophaeus* were seeded in nutrient agar and *S. dysenteriae* in Sallmonella-shigella agar. Wells were created into the centre of the plates of the seeded organisms using sterile cork-borer of 6 mm in diameter. 40 mg/ml of the extracts prepared in distilled water were introduced into the wells and allowed to stand for one hour (1 h) at room temperature for proper diffusion. All the plates were incubated at 37°C for 24 h. The zones of inhibition were measured and recorded.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the crude extract was determined using the method of Akinpelu and Kolawole (2004). Forty milligram per milliliter (0.4 g in 10 ml of distilled water) suspension of each extract was reconstituted into nutrient broth in test tubes and the 40 mg/ml suspension was used as initial concentration. Four more tubes with 5 ml of nutrients broth were setup and 5 ml of the initial concentration was used in two-fold dilution, constituting concentrations of 40, 20, 10, 5 and 2.5 mg/ml.

Normal saline was used to prepare suspensions of the microorganisms; the dilution was done continuously and incubated at 37°C for 30 min until the turbidity matched that of 0.5 McFarland's standard by visual comparison. One hundred microliters of the cell suspension was inoculated into each of the tubes with varied concentrations. All the tubes were incubated at 37°C for 24 h. The tube with the lowest concentration which had no growth of the microbes was taken as the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by sub-culturing the tubes of the MIC analyzes that did not show any growth or turbidity into plates of nutrient agar or Muller Hinton agar for *E. faecalis* and Sallmonella-shigella agar for *S. dysenteriae.* All the plates were incubated at 37°C for 24 h. The MBC was taken as the concentration of the extract that did not show any colony growth on the agar plates (Spencer and Spencer, 2004)

Table 1. The photochemical components of the extract.

Phytochemical component	Remark
Saponins	Present
Glycosides	Present
Phlobatannins	Present
Terpenoids	Present
Steroids	Absent
Cardiac glycosides	Present
Flavonoids	Present
Tannins	Present

Table 2. The Antimicrobial screening of the extract showing the zones of inhibition (MM).

Test organism	Methanol extract	Aqueous extract
S. dysenteriae	31	29
B. atrophaeus	26	24
P. aeruginosa	30	28
C. albicans	27	24
E. faecalis	28	25

Table 3. Minimum inhibitory concentration (MIC) of the extract against the microbes.

Test organism	Methanol extract (mg/ml)					Aqueousextract (mg/ml)				
	40	20	10	5	2.5	40	20	10	5	2.5
S. dysenteriae	-	-	0*	+	++	-	-	0*	+	++
B. atrophaeus	-	0*	+	++	+++	-	0*	+	++	+++
P. aeruginosa	-	-	0*	+	++	-	0*	+	++	+++
C. albicans	-	0*	+	++	+++	-	0*	+	++	+++
E. faecalis	-	0*	+	++	+++	-	0*	+	++	+++

-: No growth (No turbidity); 0*: MIC; +: Low growth; ++: Moderate growth; +++: High growth.

RESULTS AND DISCUSSION

The result of phytochemical analysis of the aqueous extracts of *A. sisalina* juicerevealed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides as shown in Table 1. The concentration of secondary metabolites varies amongst the extracts evaluated. The presence of these components in this species shows that it may have some medicinal potential. This is probably due to the fact that each of the components identified has record of one therapeutic usage or another. For instance, plants rich in saponins are known to be immune boosting and have antiinflammatory properties (Kenner and Requena, 1996).

Similarly, plants with tannins have antibacterial potentials due to their basic character which allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by Adesina et al. (2000), Ajoku et al. (2005), Dboh and Abudu (1997), and Onwuliri and Wonany (2005).

The antimicrobial screening of the extract shows varying zones of inhibition against the test organisms varying from 26 to 31 mm for methanol extract and 24 to 29 mm from aqueous extract (Table 2). It clearly indicates that the methanol extract had more activity against the test organisms. This might be that the solvent, methanol, extracted more of the active biological compounds than the aqueous extract as given by Flavia et al. (2008) and Mahida and Mohan (2007). The control test of the solvent, methanol was set and there was no effect against the tested organisms.

From Table 3, the minimum inhibitory concentration of the extract starts from 10 to 20 mg/ml. The range of the

Table 4. Minimum bactericidal concentration (MBC) of the extract against the microbes.

Test organism	Methanol extract (mg/ml)					Aqueous extract (mg/ml)				
	40	20	10	5	2.5	40	20	10	5	2.5
S. dysenteriae	-	0*	+	++	+++	-	0*	+	++	+++
B. atrophaeus	0*	+	++	+++	++++	0*	+	++	+++	++++
P. aeruginosa	-	0*	+	++	+++	-	0*	+	++	+++
C. albicans	0*	+	++	+++	++++	0*	+	++	+++	++++
E. faecalis	0*	+	++	+++	++++	0*	+	++	+++	++++

-: No growth (No turbidity); 0* = MBC; +: LOW growth; ++: Moderate growth; +++ High growth; ++++: Dense growth

concentrations was quite low as compare to research reported by Reuben et al. (2008). *S. dysenteriae* was more inhibited among the test organisms, with concentration of 10 mg/ml for the both extracts. For both the extracts; the *B. atrophaeus, C. albicans and E. faecalis* have their inhibition with 20 mg/ml.

The minimum bactericidal concentration (MBC) of the extracts as in Table 4 starts from 20 to 40 mg/ml against the test organisms. The study revealed the potentiality of the plant, *A. Sisalina* for medicinal use in the treatment of these pathogenic organisms.

Conclusion

The presence of some of the phytochemical components in *A. Sisalina* has the possibilities of being immune boosting and have antiinflammatory properties. From this, combining it with the antimicrobial screening, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts, *A. Sisalina* shows properties to be used for medicinal purposes in the treatment of some pathogenic organisms.

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