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NATURAL DRUGS

PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF METHANOL AND AQUEOUS EXTRACTS OF *AGAVE SISALANA*

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Abstract: The methanol and the aqueous extracts of the plant, *Agave sisalana* were prepared and evaluated for its phytochemical properties and antimicrobial activities. The phytochemical analysis of the preparation revealed the presence of some secondary metabolites which include: saponins, glycosides, cardiac glycosides, steroids, tannins and flavonoids. The *in vitro* antimicrobial activity of the crude methanol and aqueous extract of the *Agave sisalana* were investigated. The extract showed antimicrobial activities against the test organisms with different zones of inhibition ranging from 28–32 mm and 25–29 mm for methanol and aqueous extract, respectively. The minimum inhibitory concentration (MIC) of both the methanol and aqueous extract was between 10–20 mg/mL, and the minimum bactericidal concentration (MBC) was between 20–40 mg/mL for both extracts. The investigation indicated that the methanol extract inhibited the growth of the microbes more than the aqueous extract. The ability of the crude extracts of *A. sisalana* to inhibit the growth of the microbes is an indication of its antimicrobial potential, which may be employed in the management of microbial infections.

Keywords: *Agave sisalana*, antimicrobial activity, phytochemical properties, minimum inhibitory concentration, minimum bactericidal concentration

In history, plants have played a major role in the production of biological compounds for the formation of drugs. Their role may either become a base for the development of medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases (1). Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious and safe (2). The scientific search for new drugs from natural products remains a serious task for scientists worldwide. It is a fact that a large segment of the population in tropical countries rely on traditional medicines for their health needs (3). Over 80% of population in the developing world make use of medicinal plants extracts to provide health (4). The searches for new compounds with antimicrobial activity from plants have been the subject for intense research in recent years (5–7). This is due to the fact that plant are widely used in folk medicine to combat various diseases in human caused by pathogenic organisms (8–10). For this reason, many researchers are aiming to scientifically prove the use of plant extracts as an effective means of control of infections and body malfunctions (11, 12).

Agave sisalana, popularly known as sisal, belongs to a family known as Agavaceae. It is a monocotelydenous plant from Mexico, it spreads to semi-arid regions. Today, Brazil is the largest production of sisal for the supply of fibers (13). In the 19th century, the plant spreaded to countries in Africa like Tanzania and Kenya and Asian countries. Today it has covered some parts the tropics. *A. sisalana* is used as a rope. It has also been used for making cloths, wall coverings and carpets (14). It contains saponins, which can be found in excess in the leaves and can be used in the production of soap (15). It is used in land reclamation schemes in arid regions of the world (16). The dried flowering stems are used as waterproof thatch (15). In some parts of the northern Nigeria, the flowering stems are also used as quiver for arrows. It has also been reported that the plant has insecticidal properties; however, further details are not yet given (17). The heart of

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the plant is very rich in saccharine matter and can be eaten when baked (18). The seed ground into flour is used as a thickener in soups or used with cereal flours when making bread (19).

Medically, it has also been reported that the plant is useful in the treatment of some infectious diseases and body malfunctions. The sap is used in the manufacture of gum arabic (binding agent) and resins for pharmaceutical industries (16). The sap can also be taken internally for the treatment of diarrhea, dysentery, etc. (20). The gum from the root is used in the treatment of toothache and the root itself is diuretic and diaphoretic, it is also used in the treatment of syphilis, local inflammation and tumor (17). The plant is used internally in the treatment of indigestion, flatulence, constipation and jaundice (16).

In this work, the antimicrobial property of the plant leave extracts against some strains of microorganisms was evaluated. Thus, the phytochemical with adequate antimicrobial efficacy proved that the plant may be adapted for treatment of some infections caused by microorganisms.

MATERIALS AND METHODS

Preparation of extracts

Fresh leaves of the plant were collected at the National Research Institute for Chemical Technology (NARICT), Zaria and was identified by the Department of Botany, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna state of Nigeria.

The leave was dried at 50^{o}C using electric drier and crushed with the aid of a mechanical grinder to powdered form. This powdered plant material (250 g) was weighed and transferred into 400 mL of water and methanol, respectively. The crushed plant material was allowed to soak for two days with shaking at intervals of time to ensure proper extraction of the active ingredients from the sample according to the method reported by Harborne (21). The mixtures were then filtered on Whatman's No.1 filter paper. The methanol filterate was concentrated to dryness using rotary evaporator *in vacuo* to obtain the crude methanol extract. The aqueous extract was lyophilized to obtain a dry powder extract. The respective extracts obtained were kept in a refrigerator until use.

Test microorganisms

The test microorganisms employed for these studies include two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), two Gram negative bacteria (E*scherichia coli* and *Salmonella* t*yphi*) and one fungus (*Candida albicans*). The clinical isolates were all obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Phytochemical screening of plant extracts

The extracts were subjected to tests for secondary metabolites such as tannins, flavonoids, steroids, glycosides, cardiac glycosides and saponins. The tests were carried out using standard methods of analysis (3, 22).

Antimicrobial screening.

The antimicrobial activity of the plant extracts were determined using agar well diffusion method (23–25). The bacterial and the fungal isolates collected in prepared slants of nutrients agar were subcultured into prepared nutrients broth and incubated at 37^{o}C for 24 h and standardized to 0.5 Mc-Farland Scale (10^{8} cfu/mL) in a prepared normal saline. The cell suspensions were seeded into prepared plates of nutrients agar. Wells were then bored into the plates of the seeded organism using sterile cork-borer of 6 mm in diameter. Each of the methanol and aqueous concentrated extracts (0.4 g) were constituted into 10 mL of their respective solvents of extraction (methanol and water), making the initial concentration to be 40 mg/mL. The concentrations were introduced to fill the wells created to the bream and allowed to stand for 30 min at room temperature for proper diffusion and then incubated at 37^{o}C for 24 h in an incubator. Controls were also set up in parallel, but using the solvents of extractions only. After the 24 h incubation, the plates were observed for zones of inhibition which were recorded in millimeters (mm).

Minimum inhibitory concentration (MIC)

The determination of the MIC of the crude extracts was carried out using the test tube dilution methods (26–28). Each of the extracts was constituted by dissolving 0.4 g of the concentrates in 10 mL of nutrient broth, making the concentration to be 40 mg/mL. Five tubes of 5 mL of nutrient broth were set up, and 5 mL of the 40 mg/mL of the extracts were taken and used for two-fold dilution into the five tubes of the nutrient broth, making the concentration to be 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL. Normal saline was used to prepare a turbid suspension of the microbes, the dilution of the microorganism was done continuously in the normal saline until the turbidity marched that of 0.5 Mc-Farland's standard by

visual comparison. At that point, microorganism has concentration of about 1.5×10^8 cfu/mL. One drop equivalent to 0.1 mL of the suspension of the microbes (prepared as previously described) was transferred into the different test tubes. The tubes were incubated at 37°C for 24 h. The minimum inhibitory concentration was regarded as the lowest concentration that inhibited visible growth.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extracts was determined by using the method of Spencer and Spencer (29). The tubes of the MIC that showed no growth (no turbidity) of the microbes were sub-cultured into a freshly prepared nutrient agar plates and 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 new set of the agar plates.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis (Table 1) showed that the concentration of saponins were high (+++) in the aqueous extract while the same were moderate (++) in the methanol extract. Steroids, tannins and flavonoids were all moderately present (++) in the methanol extract, whereas in the aqueous extract only flavonoids were of moderate concentration, with steroids and tannins of low concentrations (+). Cardiac glycosides were low in concentration in the methanolic extract (+) and absent in aqueous extract.

These compounds are found to be biologically active and therefore, aid in the antimicrobial activities. These secondary metabolites exert antimicrobial activity by different mechanisms; tannins has been found to react with proline-rich protein to from irreversible complexes (30), resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their major components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (31). Therefore, these observations could support the use of this plant in herbal cure remedies. The presence of saponins lend credence to the use of this plant in managing inflammation (32) worked on steroidal extracts from medical plants which exhibited antibacterial activities on some bacterial isolates. Flavonoids have also exhibited a wide range of biological activities; such as antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-allergic and cytostatic properties (33).

Antimicrobial activity

Both the methanol and aqueous extracts showed varying degree of antimicrobial activity against the test organisms (Table 2). The methanol extract (inhibition zone 28–32 mm) was found to be more effective than the aqueous extract (inhibition zone 25–29 mm) against all the test organisms. In methanol extract, *Escherichia coli* was more susceptible than any other selected organism with zone of inhibition of 32 mm and *Salmonella typhi* showed the least susceptibility. Also for the aqueous extract, *Staphylococcus aureus* was highly susceptible to the extract (29 mm) and *Streptococcus pyogenes* showed the least susceptibility (25 mm).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) evaluations

The minimum inhibitory concentration (MIC) of both the methanol and aqueous extract against the different test organism ranged from 10–20 mg/mL (Table 3). The MIC of both the extracts against *Staphylococcus aureus, Candida albicans, Escherichia coli* and *Streptococcus pyogenes* start from 10 mg/mL; *Salmonella typhi* was inhibited by the two extracts at 20 mg/mL. Also at this concentration

Table1. The phytochemical components of the extracts.

	Methanol extract	Aqueous extract
Saponnins	++	+++
Steroids	++	+
Tannins	++	+
Flavonoids	++	++
Cardiac glycosides1	+	–

+++ = high concentration; ++ = moderate concentration; + = low concentration; – = absent

Table 2. The antimicrobial activity of the extracts showing zones of inhibition (mm)

Test organism	Methanol extract	Aqueous extract
Staphylococcus aureus	30	29
Salmonella typhi	28	27
Escherichia coli	32	26
Streptococcus pyogenes	30	25
Candida albicans	31	28

Table 3. Minimum inhibitory concentration of the extracts 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
Staphylococcus aureus	–	–	0*	+	++	–	–	0*	+	++
Salmonella typhi	–	0*	+	++	+++	–	0*	+	++	+++
Escherichia coli	–	–	0*	+	++	–	0*	+	++	+++
S*treptococcus pyogenes*	–	–	0*	+	++	–	0*	–	+	++
Candida albicans	–	–	0*	+	++	–	–	0*	++	+++

– = no growth; 0* = MIC + = light growth; ++ = moderate growth; +++ = high growth

Table 4. Minimum bactericidal concentration (MBC) of the extracts 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
Staphylococcus aureus	–	0		+	++	–	0	+	++	+++
Salmonella typhi	0	+		++	+++	0	+	++	+++	+++
Escherichia coli	–	0		+	++	0	+	++	+++	+++
S*treptococcus pyogenes*	–	0		+	++	0	+	++	+++	+++
Candida albicans	–	0		+	++	–	0	+	++	+++

– = no growth; 0* = MIC + = light growth; ++ = moderate growth; +++ = high growth

Escherichia coli and *S. pyogenes* were inhibited by the aqueous extract.

The minimum bactericidal concentration (MBC) of both extracts against the different organism ranged between 20–40 mg/mL (Table 4). The MBC of both the extracts starts from 20 mg/mL against *Staphylococcus aureus* and *Candida albicans*. Then the MBC of methanol extract against *Escherichia coli* and *S. pyogenes* also starts from 20 mg/mL. At concentration of 40 mg/mL, *Salmonella typhi* were killed by both extracts and *E. coli* and *S. pyogenes* were also killed by the aqueous extract.

CONCLUSION

The extracts of *A. sisalana* has demonstrated significant biological activity against the test pathogenic organisms and this has introduced the plant as a potential candidate for drug development for the treatment of diseases caused by these pathogens (34). Of course, there would be the need to ascertain by further studies whether any single or combination of the pure active metabolites (35) would be better, safer and more efficient in treating diseases caused by the selected pathogens than the whole plant (crude extracts) or not.

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