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Full Length Research Paper

Phtyochemical screening and comparative study of antimicrobial activity of *Aloe vera* various extracts

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The phytochemical screening and comparative study of antimicrobial activity of *Aloe vera* extracts was carried out. The phytochemical screening revealed the following metabolites: saponins, alkaloids, glycosides, tannins, protein, and flavonoids. While cardiac glyocosides and steroids were absent. The antimicrobial activities of the gel in 10% DMSO, methanol and aqueous extracts of green rind and leaf pulp were carried against some pathogenic clinical isolates, namely, *Bacillus subtilis, Staphylococcus aureus, Proteus mirabilis* and *Candida albicans.* The DMSO extract of the gel and the methanol extract of the green rind showed greater potency against tested microorganisms in the order *B. Subtilis> S. aureus> C. albicans* and *S. aureus>C. albicans>B. subtilis>P. mirabilis* respectively with different zones of inhibition ranging from 18 to 35 mm gel, 15 to 18 mm for leaf pulp methanol, 20 to 32 mm green rind methanol, also 12 to 14 mm and 14 to 23 mm for leaf pulp aqueous and green rind aqueous respectively. The minimum inhibitory concentration (MIC) of the extracts against organisms ranged from 6.25 to 25 mg/ml while the minimum bactericidal concentration (MBC) was within the range of 12.5 to 50 mg/ml. The study revealed that the green rind methanol extract and gel have greater medicinal potential against *B. subtilis*.

Key words: Antimicrobial, gel and green rind extract, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and phytochemical screening, *Aloe vera*.

INTRODUCTION

Medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases. Analysis of different species of medicinal plants for biologically active components known to have pharmacological properties have been conducted and most of the studied plants have shown antimicrobial property (Rabe and Vanstaden, 1997, Ongsakul et al., 2009; Ahsan et al., 2009).

As a result of resistance development by pathogenic organisms against antibiotics, side effects and consumers demand, scientific interest is directed to extraction, characterization of the potent active ingredients and subsequent development of drugs, herbal products as supplement (Khan et al., 2008), topical products and varieties of surfactants for internal use (Anoymous, 2006; Hamman, 2008).

Aloe plant is a native of the parts of Africa, especially South Africa's Cape Province and the mountains of tropical Africa. It is also grown in subtropical and tropical locations including South America and Caribbean. The plant is considered to belong to Liliaceae and Aloeaceaa family which has numerous species. *Aloe vera Barbadensis* Miller is the only plant that is known to have legendary medicinal reputation dating back to thousands of years ago (Anonymous, 2006). It contains treasures of nutritional and antipathogenic compounds. The composition of the plant has been a subject of considerable scientific research and further development continue to reveal new components (Reynolds, 1983).

More than 200 chemical components have been

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identified from the leave pulp and exudates of *A. vera* plant (Reynolds, 1983; Choi and Chang, 2003; Ni and Tizard, 2004).

The chemical composition of the leaf pulp and exudates was summarised to include Anthraquinones/ anthrones, carbohydrates, chromones, enzymes, inorganic compounds, mislaneous organic compounds and lipids, non essential and essential amino acids, proteins, saccharides and vitamins (Hamman, 2008). The therapeutic properties of *A. vera* gel such as anti-inflammatory effects, wound healing effects, antibacterial, antiviral, antifungal, antidiabetic, anti-neoplatic activities and antioxidant effects is the function of the polysaccharide constituent (Choi and Chang, 2003; Ni and Tizard, 2004; Ramachandra and Srinivasa Rao, 2008). Other biological and physiological activities exhibited by the *A. vera* pulp include inhibition of AIDS virus by accemannan and inhibition of the prostaglandin synthesis by anthraquinone-type compound (Hasanuzzaman et al., 2008).

The results of investigation into the antimicrobial activity of different *A. vera* species in different locations have been published individually (Agary et al., 2005; Alemdar and Agaolu, 2009; Lawrence et al., 2009; Arumkumar and Muthuselvan, 2009). The difference in the results could be attributed to the used of the plant from geographical locations with variation in their chemical composition, different processing and isolation techniques that were applied to extract the gel and gel components from the Aloe leaf. Castillo et al. (2005) evaluated the inhibitory effect of the pulp and the liquid fraction against plant pathogenic fungi and other bacteria/fungi affecting humans. The efficacy of the Aloe gel as antimicrobial agent is shown to have wide range of activity against Gram positive and Gram negative bacteria (Anonymous, 2008). Topical application of the gel has been effective in the treatment of various skin diseases including burns, cuts, eczema (Arumkumar and Muthselvan, 2009) and alleviates the advances of skin cancer by cause by sun. Oral administration has been found to be effective against stomach and intestinal disorder (Lawrence et al., 2009), accelerate the healing of mouth ulcer and aid the growth of new tissues (Anonymous, 2006). It also has the ability to stimulate microphages (Davis, 1997).

The aim of this work is to conduct a comparative study of the antimicrobial activity and also the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the pulp, gel and green rind fraction of the leaf.

MATERIALS AND METHODS

Preparation of the extracts

Fresh leaves of *A. vera* were collected from the *A. vera* farm at National Research Institiute for Chemical Technology (NARICT), Zaria. The gel was extracted from the leaves using traditional hand filleting procedure and the whole leaf was also used. The gel obtained via the hand filleting method was then lyophilized to get dry powder. The green rind which is the outer cover of the leave was also collected and dried at 50°C using Gallenhamp drying cabinet. Also the whole leaf was chopped into pieces and dried at the same temperature. All the dried parts of the leaves were grinded into powdered form using mortar and pestle. 250 g of the whole leaf powder and the green rind powder were dissolved in 300 ml each of distilled water and methanol for extraction. This process was allowed to soak overnight for proper extraction of the active ingredients at room temperature (Harbone, 1991). The mixture was then filtered using Whatman No. 1 filter paper. The solvents were evaporated using water bath at a maintained temperature to ensure proper concentration. The methanol extract of the leaf pulp, methanol extract of the green rind, aqueous extract of the leaf pulp, aqueous extract of the green rind and the dimethyl sulfoxide (DMSO) extract of gel labeled A, B, A1, B1, and C, respectively.

Test microorganisms

The test organisms selected for this work include: *Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, and Candida albicans.* These organisms were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria and transported in slants of nutrient agar to National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

Phytochemical screening of the extracts

The phytochemical screening of the crude extract was carried out in order to ascertain the presence of its secondary metabolites such as saponins, alkaloids, flavonoids, steroids, tannins, cardiac glycosides, glycosides, and proteins using standard method of analyses by Sofowara (1993).

The antimicrobial screening of the extracts

The antimicrobial screening of the plant extracts were determined using agar well diffusion method as given by Irobi et al. (1994). The organisms collected were sub-cultured into prepared normal saline and incubated at 37°C for 30 min, the concentration of each organism was increased to form a turbidity that matched with 0.5 McFarland's standard by visual comparison at which it is assumed that the number of cells is 1.5×10^8 cfu/ml. The cell suspensions were seeded into prepared plates of nutrient agar. Wells were then bored into the plates of seeded organisms using sterile cork borer of 6 mm in diameter. 50 mg/ml of the extract was constituted in distilled water and 10% DMSO for the gel as given by Ongsakul et al. (2009). This concentration was introduced into each wells and allowed to stand for 30 min at room temperature for proper diffusion. Alongside the solvent control of 10% DMSO and drug positive control (Tetracycline hydrochloride, HCl) of 50 mg/ml were also set up. All the plates were incubated at 37°C for 24 h.

Minimum inhibitory concentration (MIC)

The MIC of the crude extracts was determined using the method described by Akinpelu and Kolawale (2004). 50 mg/ml of each of the extracts were reconstituted into nutrient broth in test tubes and the 50 mg/ml was taken as the initial concentration. Four more tubes of 5 ml nutrient broth were set up and 5 ml of 50 mg/ml of the extract was taken and used for two-fold dilution of the four tubes of nutrient broth forming concentrations of 50, 25, 12.5, 6.25 and

Table 1. The phytochemical screening of the extracts.

Components	B1	A1	C
Saponins	+++	++	+
Glycosides	+	+++	++
Steroids	-	-	-
Flavonoids	+	+	++
Alkaloids	++	+++	++
Tannins	+	+	+
Cardiac glycosides	-	-	-

B1 = Aqueous extract of the green rind, A1 = Aqueous extract of the leaf pulp, C = Gel.

Table 2. The antimicrobial screening of the extracts of *Aloe vera* zones of inhibition of the extracts against the selected microbes (mm).

Test organism	A	B	A1	B1	C
B. subtilis	15	23	14	15	35
S. aureus	18	32	0	23	27
C. albicans	16	25	12	14	18
P. mirabilis	0	20	0	0	0

KEY: A = Methanol extract of the leaf pulp, B1 = Aqueous extract of the green rind, A1 = Aqueous extract of the leaf pulp, C = Gel, B = Methanol extract of the green rind.

3.125 mg/ml.

Normal saline was used again to prepare turbid suspensions of the microbes; the suspended cells were incubated at 37°C for 30 min until the turbidity matched with the prepared 0.5 Mcfarland's standard by visual comparison, at that point the colony forming unit of the cells is assumed to be 1.5×10^8 cfu/ml. 0.1 ml 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 has no growth (turbidity) of the microbes was taken to be minimum inhibitory concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extract against the microbes was determined using the method of Spencer and Spencer (2004). The tubes of the MIC that showed no growth of the microbes were sub-cultured by streaking using sterile wire loop on nutrient agar plates. The plates were incubated at 37°C for 24 h. The MBC was taken as the lowest concentration of the extract that showed not any colony growth on the agar plates.

RESULTS AND DISCUSSION

The result of the phytochemical screening revealed the presence of some active compounds which include saponins, glycosides, flavonoids, alkaloids and tannins (Table 1).

Secondary metabolites identified in plant material have been reported as having inhibitory action against pathogenic microorganisms (Freeman and Beattle, 2008; Xiao-tian and Wei-shuo, 2006). The presence of the above named secondary metabolites in the various *A. vera* gel and extracts could be attributed to the inhibitory activities observed in the antimicrobial tests conducted using pathogenic organisms.

Glycosides and alkaloids were highest in the whole leaf *A. vera* gel when compared to the lyophilized gel and the green rind while saponins had the highest value in the green rind. Steroids and cardiac glycosides indicated negative result in all the extracts. Igbinosa et al. (2009) reported that these secondary metabolites exert antimicrobial activity through different mechanisms: Tannins have been found to form irreversible complexes with proline-rich protein resulting in the inhibition of cell protein synthesis.

Herbs that have tannins as the main components are astringent in nature and are used in the treatment of intestinal disorder. The presence of tannins supports the traditional medicinal use of this plant in treatment of different ailments.

Alkaloids which are one of the largest groups of phytochemicals in plant have amazing effects on humans and this has led to the development of pain killer. The presence of saponins in plant has supported the management of inflammation. Flavonoids have activities like antimicrobial, antioxidant anti-inflammatory, analgesic and anti-allergic properties.

The antimicrobial activity of the extracts against the test organisms with varying zones of inhibition ranging from 12 to 35 mm has revealed the antimicrobial potency of this plant. *B. subtilis* was more affected by extract C as shown in (Table 2 and Figure 1). This agreed with report

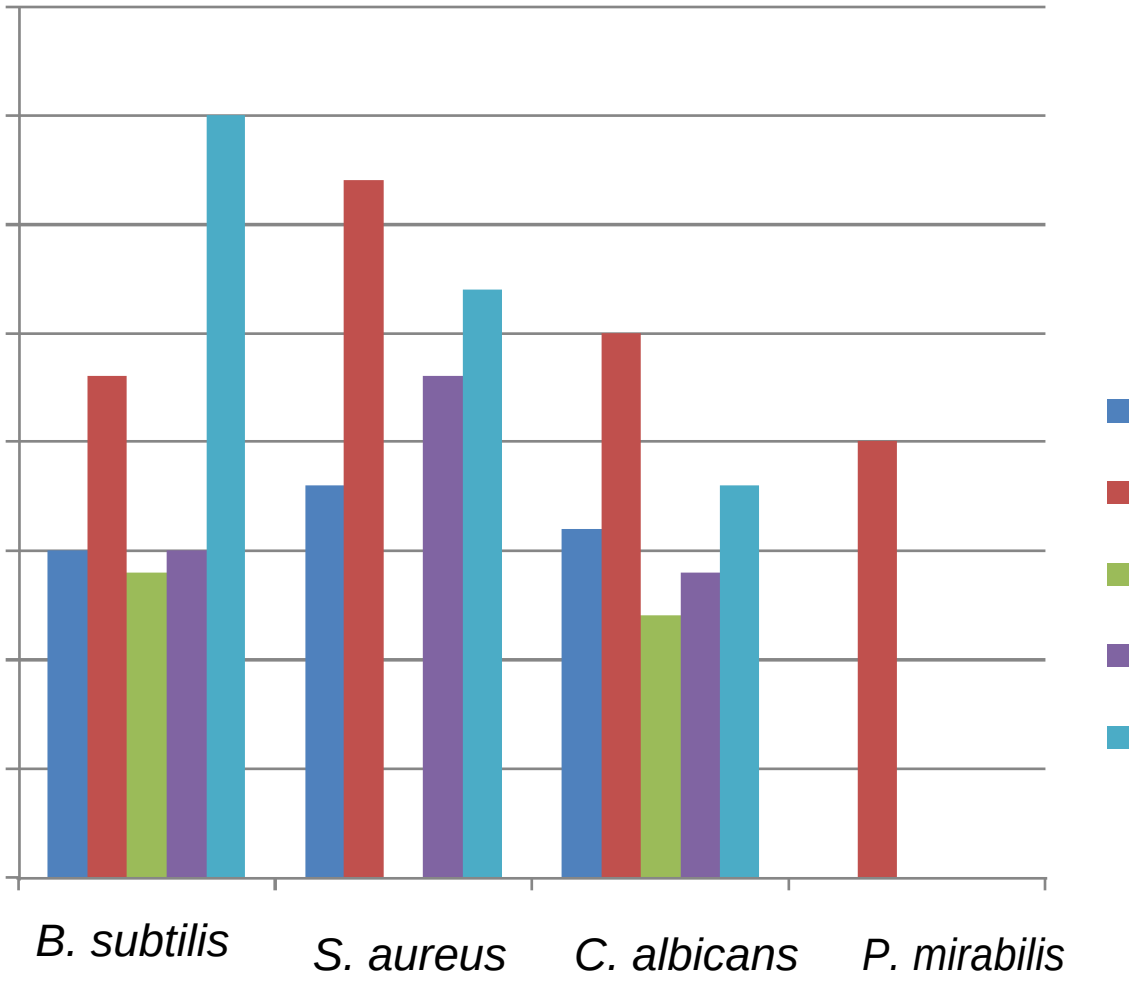

Figure 1. Histogram respresenting the zones of inhibition of the extracts against the test organisms.

of Agary et al. (2005). Also in this work *S. aureus* was also immensely affected, *C. albicans* was affected by extracts B and C. Only extract B had effect on *P. mirabilis*.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were in the range of 6.25 to 25 mg/ml and 12.5 to 50 mg/ml respectively (Tables 3 and 4). The activities of *B. subtilis and C. albicans* in extracts A, B, A1 and B1 were inhibited at 25 mg/ml while in extract C the minimum concentration that showed no growth of B. *subtilis* and C. *albicans* are 6.25 and 25 mg/ml respectively. The growth of S. *aureus* in extracts A, B, B1 and C was inhibited at 25, 12.5 and 6.25 mg/ml concentrations respectively. Below the minimum inhibitory concentrations growth of the microbes was observed ranging from light growth through moderate growth and to high growth as seen in (Table 3).

This made us to understand the ability of the plant to be used in the treatment of diseases caused by the above affected organisms. The growth of *C. albicans* was also inhibited by the various *A. vera* extracts with varying zones of inhibition. Many different clinical forms of candidiasis are known involving primarily the mucosa surface (thrush gastrointestinal or urogenital tract) and deep-seated infections such as candidaemia or meningitis. Candida vaginitis is a common infection during pregnancy (Cheesbrough, 1984). The results of inhibiting effect on *C. albicans* also established that the *A. vera* gel and leaf rind, though share certain components, are distinct from one another (Foster, 1999). The negative control using the 10% DMSO

Table 3. Minimum inhibitory concentration (MIC) of the extracts against the microbes (in mg/ml).

Test organism	A			B							A1					B1									
	50	25	512.	50	50	50	50	50	256.	1253.	50	25	512.	256.	1253.	50	25	512.	256.	1253.	50	25	512.	256.	1253.
B. subtilis	-	0*	+	-	-	-	-	-	++	+++	-	0*	+	++	+++	-	0*	+	++	+++	-	-	-	0*	+++
S. aureus	-	0*	+	-	-	-	-	-	+	++						-	-	0*	+	++	-	-	-	0*	+
C. albicans	-	0*	+	-	-	-	-	-	++	+++	-	0*	+	++	+++	-	0*	+	++	+++	-	0*	+	++	+++
P. mirabilis									++	+++															

Key: = No growth (turbidity), ++ = moderate growth, 0*= MIC, +++ = high growth, + = light growth.

Table 4. Minimum bactericidal concentration (MBC) of the extracts against the microbes (in mg/ml).

Key: = Now growth, ++ = moderate growth, 0* = MBC +++ = high growth, + = light growth ++++ = numerous growth.

Table 5. The set of controls for the antimicrobial screening showing zones of inhibition in mm.

Test organism	Negative (solvent) control, 10% $DMSO_4$	Positive(drug) control, Tetracycline (50 mg/ml)
B. subtilis	0	50
S. aureus	0	41
C. albicans	0	36
P. mirabilis	0	37

had no effect on the selected organisms. The positive control which was Tetracyline HCl had wide effect against the organisms the crude extracts as indicated in (Table 5).

Conclusion

The screening of the plant has revealed its potency in the treatment of pathogenic infections that may be caused by these pathogens. We, therefore, suggest the isolation and possible characterization of the bioactive constituent(s) from the extracts of the plant as a possible

antimicrobial agent, especially the gel and the green rindmethanol (C and B) extracts.

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