International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 2(10) pp. 382-386, November 2011
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

Phytochemical screening and a comparative study of antibacterial activity of *Aloe vera* green rind, gel and leaf pulp extracts

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Accepted 05 October, 2011

A phytochemical and comparative study of the antibacterial activity of Aloe vera extracts were carried out. The phytochemical screening revealed the presence of bioactive compounds such as saponins, alkaloids, flavonoids, tannins, glycosides, and proteins, with absence of cardiac glycosides and sterols in all investigated extracts. According to the antibacterial activity results, *Escherichia coli* was sensitive to all extracts while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were resistant to methanol and aqueous leaf pulp extracts, *Salmonella typhi* was sensitive to gel and the green rind aqueous (GRA) extract . The GRA and gel exhibited high activity against the assayed bacteria. The MIC value ranged from 6.25 to 25mg/ml and the MBC ranged from 12.5 to 50mg/ml. Among the assayed extracts, the gel exhibited great potential against the bacteria tested in this study.

Keywords: Antibacterial, phytochemical, MIC, MBC and gel.

INTRODUCTION

Medicinal Plants are used by local communities since centuries (Shinwari, 2010). Aloe vera is an ornamental plant and has been used for many centuries due to its curative and therapeutic properties. In the pharmaceutical industry, it has been used in the synthesis of topical products such as ointments and gel preparations and also in the development of tablets and capsules. In food industry it is used as source of functional foods or parts of the ingredients in other food products (Hamman, 2008). The plant has also been reported to have anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant, antimicrobial, skin hydration, wound healing and hepatoprotective effects. The antimicrobial activity of *A. vera* plant extracts against *Bacillus (subtilis, Staphylococcus aureus, Proteus mirabilis* and *Candida albicans* has been reported (Yebpella *et al*; 2011; Cock, 2008). It is reported to contain vitamins, minerals, mono- and polysaccharides, organic acids, and enzymes (Arunkumar and Muthuselvam, 2009).

Plants and herbs have taken important role in the treatment of different ailments caused by pathogens and non-pathogens. Infections caused by pathogenic microorganisms have in high mortality in the developing world. These infections may be invasive and are increasing due to the increase of their incidence in the hospitals (Santos, *et al;* 2009). Igbinosa *et al* (2009) reported that traditional medicine using plants extracts continue to provide health coverage for over 80% of the world population especially in the developing world (Hammuel *et al;* 2011). The used of medicinal plants and herbs for the treatment of pathogenic and non pathogenic diseases has also been encouraged by World Health Organization (WHO, 1995).

The plant *Aloe vera* is a wonder medicinal plant and it's belongs to family of Liliaceae and Aloeaceaa having numerous species of about 400. *Aloe vera* gel is made of

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99.3% water, the remaining 0.7% is made of solids with glucose and mannose as the major constituents in the leaf (Agarry *et al;* 2005). These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product. *Aloe vera* Barbadensis Miller is the only known to have legendary medicinal records dating back thousands of years ago (Yebpella *et al;* 2011). Although *A. vera* has been studied for its medicinal importance, there is a need to separately assess the different parts of the leaf for antibacterial activity. This work was conducted to provide with information on the comparative antibacterial activity of the leaf pulp and green rind extracts as well as the gel using methanol and water as solvents of extraction.

MATERIALS AND METHODS

Preparation of the extracts

Fresh leaves of *A. vera* were collected from the *A. vera* farm at National Research Institute for Chemical Technology (NARICT), Zaria. The gel was extracted from the leaves using traditional hand filleting procedure (Anonymous, 2006) and then lyophilized. The green rind which is the leaf outer skin was collected and dried at 50^{o}C using electric drier. The leaf pulp was chopped into pieces and dried at the same temperature. All the dried parts of the leaves were grinded into powder using mortar and pestle. An amount of 250g of the leaf pulp and green rind powder were separately macerated in 300ml of aqueous and methanol for extraction and allowed overnight (Harbone, 1991). The mixture was then filtered using Whatman No. 1 filter paper. The solvents were evaporated using water bath at 40^{o}C to ensure proper concentration.

Test microorganisms

The antibacterial assay was carried out using: - *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa,*and *Klebsiella pneumoniae.* These species of bacteria were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria and transported in slants of nutrient agar and MacConkey agar.

Phytochemical screening of the extracts

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

Antimicrobial activity

The antibacterial activity was assessed by agar well diffusion method (Irobi *et al* 1994; Shinwari et al. 2009). The dried extracts were reconstituted with sterile distilled water and 10% dimethylsulfoxide DMSO) for the gel as given by Ongsakul *et al* (2009) to obtain a stock solution of 50mg/ml from which concentrations of 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml were prepared using two-fold method of dilution. Prepared broth culture of each bacteria strain was adjusted to turbidity, equivalent of 0.5 McFarland standard at which the number of cells is assumed to be 1.5 x 10^{8}cfu/ml. The adjusted broth cultures were swabbed onto nutrient agar MacConkey agar for *klebsiella pneumoniae* and Salmonella-Shigella agar for *Salmonella typhi* plates using sterile cotton swab. Wells were bored into each of the plates using sterile cork borer of 6mm in diameter. 0.1ml of each of the extracts was introduced into wells using sterile automatic pipette. The extracts were allowed to diffuse at room temperature for 1hour. All the plates were incubated at 37^{o}C for 24 hours and the results of the zone of inhibition were recorded.

Minimum Inhibitory Concentration (MIC)

The MIC of the crude extracts was determined using the method described by Akinpelu and Kolawale (2004). 50mg/ml of each of the extracts were reconstituted into nutrient broth in test tubes and the 50mg/ml was taken as the initial concentration. Four more tubes of 5ml nutrient broth were set up and 5ml of 50mg/ml of the extract was taken and used for two-fold dilution of the four tubes of nutrient broth forming concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml.

Normal saline was used again to prepare turbid suspensions of the microbes; the dilution was done continuously and incubated at 37^{o}C for 30 minutes. Until the turbidity matched that 0.5 Mcfarland's standard by visual comparison. At that point the of cells is assumed to be 1.5 x 10^{8}cfu/ml. 0.1ml of the cell suspension was inoculated into each of the tubes with the varied concentrations of extracts. All the tubes were incubated at 37^{o}C for 24 hours. The tube with the lowest concentration which has no growth (turbidity) of the microbes was taken to be the 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

Table 1: The Phytochemical Screening of the Extracts

Components	Green Rind Aqueous	Leaf Pulp Aqueous	Gel
Saponins	+++	++	+
Glycosides	+	+++	++
Sterols	-	-	-
Flavonoids	+	+	++
Alkaloids	++	+++	++
Tannins	+	+	+
Proteins	++	+	-
Cardiac glycosides	-	-	-

Table 2: The antibacterial screening of the of *Aloe vera* extracts

Test Organism	Leaf Pulp Methanol	Green Rind Methanol	Leaf Pulp Aqueous	Green Rind Aqueous	Gel
Escherichia coli	S	S	S	S	S
Klebsiella pneumonia	R	S	R	S	S
Salmonella typhi	R	R	R	S	S
Pseudomonas aeruginosa	R	S	R	S	S

Key: R= Resistant, S= Sensitive

Table 3: Zones of inhibition of the extracts against the selected Bacteria (mm)

Test Organism	Leaf Pulp Methanol	Green Rind Methanol	Leaf Pulp Aqueous	Green Rind Aqueous	Gel
Escherichia coli	20	34	11	25	24
Klebsiella pneumonia	0	31	0	17	19
Salmonella typhi	0	0	0	19	20
Pseudomonas aeruginosa	0	18	0	15	16

Table 4: Minimum Inhibitory Concentration (MIC) of theExtracts against the Microbes (in mg/ml)#

Test Organism	Leaf Pulp Methanol					Green Rind Methanol					Leaf Pulp Aqueous					Green Rind Aqueous					Gel				
	50	25	12	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125
Escherichia coli	- +++		0*	+	++	- +		-	-	0*	- +++		0*	+	+	- ++		-	0*	+	- ++		-	0*	+
Klebsiella Pneumoniae						- ++		-	0*	+						- +++	0*	+	++		- ++		-	0*	+
Salmonella typhi																- +++	0*	+	++		- ++		-	0*	+
Pseudomonas aeruginosa						- +++		0*	+	++						- +++	0*	+	++		- +++		0*	+	++

KEY: - = No growth (turbidity) ++ = Moderate growth
0* = MIC +++ = High growth
+ = Light growth

nutrient agar plates, MacConkey agar plate for *Klebsiella pneumoniae* and Salmonella- Shigella agar plate for *Salmonella typhi.* The plates were incubated at 37^{o}C for 24 hours. The MBC was taken as the lowest concentration of the extract that showed not any colony growth on the agar plates.

Table 5: Minimum Bactericidal Concentration of the Extracts against the Microbes (in mg/ml)

Test Organism	Leaf Pulp Methanol					Green Rind Methanol					Leaf Pulp Aqueous					Green Rind Aqueous					Gel				
	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125
Escherichia coli	0* ++++	+	++		+++	-	0* ++++	+	++	+++	0* ++++	+	++		+++	-	0* ++++	+	++	+++	-	-	0*	+	++
Klebsiella Pneumoniae						0* ++++	+	++		+++						0* ++++	+	++		+++	-	0*	+	++	+++
Salmonella typhi																0* ++++	+	++		+++	- ++	0*	+	+ +	+
Pseudomonas aeruginosa						0* ++++	+	++		+++						0* ++++	+	++		+++	0* ++++	+	++		+++

KEY: - = Now growth ++ = Moderate growth
0* = MBC +++ = High growth
+ = Light growth ++++ = numerous growth

RESULTS

DISCUSSION

The phytochemical analysis (Table: 1) of the extracts revealed the presence saponins, glycosides, alkaloids, tannins, and flavonoids. Cardiac glycoside was not found in all the extracts. The presence of these biologically active compounds in the extracts has made the plant to be known of its medicinal use especially for antimicrobial activity against pathogenic organisms. Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders (Igbinosa *et al*, 2009). Alkaloid has also been reported to be a pain killer and saponin has managing effect against inflammation (Igbinosa *et al*, 2009; Hussain *et al*; 2009). Flavonoid is also important against inflammation and microorganisms.

The antibacterial screening of the *Aloe vera* extracts against the choose organisms showed zones of inhibition in different variations which range from 11-34mm. The gel and green rind aqueous extract had effect against all the bacteria ranging from 15-25mm. The bacteria were not sensitive to the leaf pulp methanol and the leaf pulp aqueous extracts except *Escherichia coli* with zones of inhibition of 20mm and 11mm respectively. All the extracts had activity against *E. coli*, *Salmonella typhi* was resistant to leaf pulp methanol, leaf pulp aqueous and green rind methanol extracts. The green rind aqueous and the gel had effect against all the selected bacteria. The negative control (DMSO) showed no effect against the bacteria, and the result of the positive control (tetracycline, HCl) almost agreed with that of green rind methanol extract against *E. coli* and *Klebsiella pneumoniae.* The inhibitory effect of the leaf rind and gel of the plant on the growth of *P. aeruginosa* gives an explanation of its reputation as a healing plant for burns (Agarry et al; 2005).

The minimum inhibitory concentration (MIC) of the extracts against the bacteria ranged from 12.5-25mg/ml and the minimum bactericidal concentration (MBC) ranged from 12.5-50mg/ml. the MIC and MBC of the gel had wide effect against *E. coli* with concentration of 12.5mg/ml, *K. pneumoniae* and *S. typhi* with concentration of 25mg/ml.

The results of the effects of all the five (5) extracts used for the study showed that the green rind methanol green rind aqueous extracts and the gel had effect against the bacteria in that order the leaf pulp aqueous and its methanol extract had least effects against the selected for this study.

CONCLUSION

Therefore, the gel and the green rind aqueous in this study have introduced the plant's parts as potential in the manipulation and development of drugs for the treatment of diseases caused by these pathogens.

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