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ANTIBACTERIAL ACTIVITY OF *ALOE VERA* PLANT EXTRACT AGAINST *SALMONELLA TYPHI*

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ABSTRACT

The antibacterial activity of the methanolic and ethanolic extracts of Aloe vera plant against Salmonella typhi was investigated, using agar diffusion technique. The ethanolic extract had zones of inhibition ranging from 7mm - 14mm at concentration of 200mg/ml - 500mg/ml and the methanolic extract had zones of inhibition ranging from 6.8mm - 15mm at concentration of 200mg/ml - 500mg/ml. The different zones of inhibition recorded for the two extracts were statistically insignificant at ($P<0.05$). The minimum inhibitory concentration (MIC) of the ethanolic and methanolic extracts was 200mg/ml while the mean minimum bactericidal concentration (MBC) of the two extracts was 250mg/ml. Phytochemical components of the Aloe vera plant extract include; anthraquinone, alkaloids, saponins, tannins, balsams and flavonoids. The result showed that Aloe vera has antibacterial activity on Salmonella typhi and may be use in the production of drugs against infection associated with the Salmonella typhi.

KEYWORDS: *Salmonella typhi, Antibacterial,;Aloe vera, Extract, Concentration, Inhibition,Methanolic, Ethanolic*

INTRODUCTION

Antibiotics provide the main basis for the therapy of microbial infection. High genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing resistance. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases (Arunkumar and Muthuselvan, 2009). This and other problems such as toxicity of antimicrobial drugs on the host tissue triggered interest in the search of new antimicrobial substances/drugs of plant origin . Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new antimicrobial substances

Aloe vera is a stemless or very short-stemmed succulent plant growing to 60-100cm (24-39 inch) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces . The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90cm (35 inch) tall, each flower pendulous, with a yellow tubular corolla 2-3cm (0.8-1.2 inch) . Like other *Aloe* species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (Alendar *et al.*, 2009).

Aloe vera has been widely grown as an ornamental plant. The species is popular with modern gardeners as a putatively medicinal plant and due to its interesting flowers, form and succulence. This succulence enables the species to survive in areas of low rainfall, making it ideal for rockeries and other low-water use gardens . The species is intolerant to very heavy frost or snow and relatively resistant to most insect pests . In pots, the species requires well-drained sandy potting soil and bright sunny conditions; however, in very hot and humid tropical climates, *Aloe vera* plants should be protected from direct sun and rain, as they will burn and/or turn mushy easily under these conditions . The use of a good-quality commercial propagation mix or pre-packaged "cacti and succulent mix" is recommended, as they allow good drainage (Alemdar *et al.*, 2009). Potted plants should be allowed to completely dry prior to re-watering. When potted aloes become crowded with "pups" growing from the sides of the "mother plant," they should be divided/removed and re-potted to allow for further growth and help prevent pest infestations.

The natural range of *Aloe vera* is unclear, as the species has been widely cultivated throughout the world. Naturalized strands of the species occur in the southern half of the Arabian peninsula, through North Africa (Morocco, Mauritania, Egypt) as well as Sudan and neighbouring countries, along with the Canary, Cape Verde, and Madeira Islands (Alendar *et al.*, 2009). The species was introduced to China and various parts of southern Europe in the 17th century and its also found growing in temperate and tropical regions of Australia, Barbados, Belize, Nigeria, Paraguay and the United State of America (Alendar *et al.*, 2009).

MATERIALS AND METHODS

Sample Collection

The *Aloe Vera* leaves were collected from a fully matured plant in the garden at Chanchaga Minna, Niger State Nigeria. It was then transported in a clean polythene bag to the laboratory for extraction of active components.

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Extraction
The leaves were washed with distilled water mixed with chlorine thoroughly to remove dirt particles. The top and bottom skin layer of the leaves were sliced off using a sterile knife and the sides of the leaves were also removed. The thin greenish layers of the leaves were removed to expose the gel. Then the gel was carefully scoped out into a clean beaker.

The extract was stored in the freezer at 4°C for 24hours to freeze dry the *Aloe vera* extract. The frozen gel was then weighed to 200g and dissolved in 400ml of ethanol and methanol solvents respectively. These was allowed to stand for 24hours for proper dissolution. The mixtures was filtered and steam dry to solid extract separately. The extracts of both solvents were reconstituted into different concentrations (132mg/ml, 147mg/ml, 200mg/ml, 250mg/ml and 500mg/ml) (Arunkumar and Muthuselvan, 2009).

Test organism
The test organism used was *Salmonella typhi* and was obtained in a stock culture from the Department of Microbiology, Federal University of Technology, Minna.

Standardization of organism
To check for the viability of the organism, some tests were conducted such as triple sugar iron test, catalase test, oxidase test and gram staining test.

Subculture on Salmonella and Shigella agar (SS agar)
A loop full of the test organisms was sub-cultured on Shigella and Salmonella agar (SS agar) then incubated for 24hours. Growth and colour of colonies was observered after incubation period.

Triple sugar iron (TSI) Test
Using a sterilized needle, the test organisms cultured on SS agar was taken and streaked on a slant of the TSI agar in a testube and stabbed to create a butt 2-3 times. The cap of the slant bottle was closed loosely and incubated at 370C for 24hours. Observation was recorded after incubation .

Catalase Test
A loop of the test organism was taken from the stock culture in a nutrient agar and placed on the glass slide. A drop of 3% hydrogen peroxide was placed on the organism. Gas production in the form of bubbles and darkening of medium indicates a positive result .

Oxidase Test
A piece of filter paper was wet with the oxidase reagent (tetramethyl-p-phenylene-diamine-dihydrochloride). A loop of the organism was placed on the wet piece of paper and smeared properly. Within 30 seconds intense colouration was noticed.

Grams staining
A smear of the test organism was made on a sterile grease free glass slide and passed through flame to fix. It was stained with crystal violet solution (primary dye) for sixty (60) seconds and washed with distilled water. It was further flooded with iodine solution (mordant) for 30 seconds and washed with distilled water. It was decolorized with alcohol in drops until all free blue colour was removed after which safranine (counter stain) was applied for 60 seconds. After which it was examined under x 100 manificaiton of the microscope .

Preparation of extracts concentrate
For each of the extract, one (1) gram of the extract was measured and dissolved in 2ml, 3ml, 4ml, 5ml, 6ml and 7ml of sterile water then converted to milligram per mil of dilution. After the conversion, 132mg/ml, 147mg/ml, 200mg/ml, 250mg/ml, 333mg/ml and 500mg/ml were used for this research.

Phytochemical Screening
The *Aloe vera* extract was screened for Anthraquinone, Tannin, Saponins, Steroids, Resuna, Carbohydrate, Alkaloids, Balsams, flavonoids, terpenes, cardiac glycosides (Alendar and Agaoglu, 2009).

Antibiotic susceptibility testing. (Cup plate method was used)
Nutrient agar medium was prepared and was dispensed into six (6) petric dishes and was allowed to set. The test organism (*salmonella typhi*) was inoculated on the media dispensed in the plates. A sterile cork borer (4mm) was used to bore 3 holes on each plate. Zero point five (0.5) ml of the different concentrates of the plant extracts that is; (143, 167, 200, 250, 333 and500mg/ml) were dispensed into the wells on the different plates and left for 20minutes for proper diffusion of the extract into the media. The zones of inhibition were measured after 24hours incubation. This was repeated for both the methanol and ethanol extracts (Mittal *et al.*, 2010).

Determination of Minimum Inhibitory Concentration (MIC)
One 1ml of the various concentration of the plant extract ; (500mg/ml, 333mg/ml, 250mg/ml, 200mg/ml, 167mg/ml and 143mg/ml) was serially diluted in 2ml of sterile nutrient broth in sterile testubes and a loop of the test organism (*Salmonella typhi*) added into the broth. This was incubated at 37°C for 24hours. The turbidity of the media was observed after 24hours which signifies the growth of organism in the media (Alendar *et al.*, 2009)

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Minimum Bactericidal Concentration (MBC)

From the result obtained from the (MIC) the broth that produced no growth were subcultured each onto a fresh nutrient agar and incubated at 37°C for 24hours (Alendar *et al.*, 2009).

RESULTS

The phytochemical components present in the *Aloe vera* extract include; Anthraquinone; Alkaloids, Saponins, Balsams, Flavonoids and Tannins (Table 1). Out of six different concentration of the ethanolic extract of *A. vera* (132, 167, 200, 250, 333 and 500mg/ml) only four (4) concentration (200, 250, 333 and 500mg/ml) had activity ranging from 4.47±0.29-13.20±0.61 against *Salmonella typhi* (Table 2).

Out of six difference concentration of the ethanolic extract of *A. vera* (132,167,200,250,333 and 500mg/ml) only four (4) concentration (200,250,333 and 500mg/ml) had activity ranging from 7.13±0.18mm-13.07±11.60mm against *Salmonella typhi* (Table 3). The minimum inhibitory concentration (MIC) of both methanolic and ethanolic extracts of *Aloe vera* on *Salmonella typhi* was 200mg/ml while the minimum bactericidal concentration (MBC) was 250mg/ml (Table 4).

Table 1: Phytochemical components of *Aloe vera* extract

S/N	Phytochemical component	Remark
1	Anthraquinone	+
2	Saponins	+
3	Alkaloids	+
4	Balsams	+
5	Flavonoids	+
6	Tannins	+

Key: + = Present

Table 2: Susceptibility test for ethanolic extract of *A. vera* against *S. typhi*

Concentration of extract mg/ml	Number of zone of inhibition measured 1	2	3	Mean
143mg/ml	0.00	0.00	0.00	0.00
167mg/ml	0.00	0.00	0.00	0.00
200mg/ml	7.4mm	7mm	8mm	7.47±0.29
250mg/ml	10mm	10.2mm	11mm	10.40±0.31
333mg/ml	12.1mm	12.0mm	12.5mm	12.20±0.15
500mg/ml	13.6mm	14mm	12mm	13.20±0.61

N.B: mean carrying the same superscript do not posses significant difference ($p<0.05$) from each other ± standard error of the mean.

Table 3: Susceptibility test for methanol extract of *A. vera* against *S. typhi*

Concentration of extract mg/ml	Number of zone of inhibition measured 1	2	3	Mean
143mg/ml	0.00	0.00	0.00	0.00
167mg/ml	0.00	0.00	0.00	0.00
200mg/ml	6.8mm	7.2mm	7.4mm	7.13±0.18
250mg/ml	11mm	10.5mm	12mm	11.17±0.44
333mg/ml	12mm	12.5mm	11mm	12.17±0.73
500mg/ml	13.2mm	15mm	11mm	13.07±11.60

N.B: mean carrying the same superscript do not posses significant difference ($p<0.05$) from each other ± standard error of the mean.

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Organism	MIC (mg/ml)	MBC (mg/ml)
S. typhi	Methanolic extract 200	250
	Ethanolic extract 200	250

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DISCUSSION

In this study the phytochemical compounds found in both the ethanolic and methanolic extracts of *Aloe vera* were Anthraquinones, Flavonoids, Saponins, Tannins and Balsams (Table 1). Study by Alemdar *et al.*, (2009) reported the presence of phytochemical components such as Anthraquinones, Flavonoids, Saponins, Tannins and Balsams in ethanolic and methanolic extracts of *Aloe vera* and also reported that the phytochemical components may be involved in the antibacterial activities of the extract on *S. typhi.*

The ethanolic and methanolic extracts of *Aloe vera* had inhibitory effects against *Salmonella typhi* at high concentrations (Table 2 and 3). The results in this study revealed that the highest zones of inhibitions of both methanolic and ethanolic extracts was at 500mg/ml and least at 200mg/ml while no zones of inhibitions was noticed in the 132mg/ml and 147mg/ml concentrations of the ethanolic and methanolic extract respectively (Table 2 and 3). This could be attributed to synergistic action by the active ingredients in the *Aloe vera* extracts when presence in large concentration, as earlier reported by Alendar *et al.* (2009). The zones of inhibitions for the methanolic and ethanolic extracts of *A. vera* on *S. typhi* were analyzed statistically using ANOVA and revealed that at $p<0.05$ no significant difference between the values was noticed.

The MIC value for the ethanolic and methanolci extracts of *A. vera* plant was 200mg/ml. this may be due to the presence of similar phytochemical components in both plant extract. This result is in agreement with the report by Arunkumar and Muthuselvan (2009) who reported similar MIC values for ethanolic and methnolic extracts of *A. vera.*

The MBC of the ethanolic and methanolic extracts was at 250mg/ml. this may be due to the phytochemical components present in both plant extracts. Deng et al., (2003) and Cummings et al. (2010) performed similar work on *S. aureus, E. coli, S. typhi and P. aeroginosa* and reported that *A. vera* extract of ethanol origin had an MBC of 250mg/ml. This study agreed with the previous reports. The result of this study is an indication that methanolic and ethanolic *Aloe vera* extract have therapeutic properties against *Salmonella typhi.* Further investigation be conducted on *Aloe vera* extract to ascertain the role of the various active components on *Salmonella typhi.*

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