Antimicrobial Properties and Nutritional Composition of *Aspilia africana* C.D. Adams

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Abstract

The antimicrobial and nutritive studies of Aspilia africana have been studied. The plant has a moisture content of 70%. Crude protein content is 3.31% measured at dry wet. The crude lipid of the plant was 0.37. The crude fibre content of the plant was 7.1% and crude carbohydrate content 78.15. The plant also contained quantities of tannin, oxalate, phytate, hydrocyanin as 4.57, 9.41, and 1.36% respectively. The presence of many elements is indicated in the plant with very low concentrations of copper, cobalt, zinc, phosphorus, lead. The plant is also rich in alkaloid, hydrolysable tannins, saponins, cardiac glycosides as phytochemical components. The methanol, ethanol and the chloroform extracts shows more zones of inhibition to the microorganisms than the aqueous and soxhlet extracts. The plant is used by herbivores mainly as food and it had been used locally for treating ear infections.

Keywords: *Aspilia africana*, antimicrobial, nutritive studies

Introduction

Aspillia africana C.D. Adams (Compositae) is a tropical shrub widely distributed across tropical Africa. In Nigeria it is commonly known as yurinyun by the Yorubas, orangila by Igbos, tozalin by Hausas and Edemedong by Efiks (Iwu, 1993). *Aspilia africana* is of high economic and medicinal importance due to it active roles in wound healing, treatment of rheumatic pains etc. The potentials of the leaves of the haemorrhage plant, *Aspilia africana* in wound care was evaluated using experimental models. *A. africana* is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores, in the treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies from the eyes. It is commonly used to feed livestock particularly sheep and goats. It is believed to have nutritive and medicinal values. Many people and livestock are suffering from malnutrition and diseases due to deficiencies of major food groups such as proteins, fats, carbohydrates and vitamins. The effect of malnutrition includes poor growth rate, decreased resistance to diseases, mental retardation, and lethargy in extreme cases starvation and death may occur (Skellern and Roger, 1977). The plant *Aspilia africana* amongst other weed was reported to be a source of protein although the quantity is not sufficient for both human and livestock demands (Umoh and Oke, 1974). Moreover, *Aspilia africana* is used locally to treat ear infections and stomach ailments. The phytochemistry of any plant part is concerned with the enormous variety of organic substances that are accumulated by the plant, and also brings in focus the chemical structures of these substances. Page, *et al* (1992) investigated the presence of fatty acid in the oil of seeds of *A. africana* and the presence of diterpenes, kaurenoic and grandiflorenic acids from the leave. The study is aimed at examining the nutritional component of *Aspilia africana* as well as assessing the antimicrobial activities of the plant.

Materials and methods

Samples collection

The plants parts were obtained in Staff Quarters, University of Calabar. Already isolated and identified specimens were collected from the University of Calabar Teaching Hospital, Chemical Pathology and Microbiology Labs.

Antimicrobial sensitivity test of the extracts

To test for antimicrobial sensitivity, one colony of each test organism was subcultured in nutrient broth with a sterile wire loop and incubated at 37^0C for 6 hours, to ensure that the bacteria were in logarithmic phase of growth. The broth containing the test organism was then inoculated on Mueller Hinton agar cooled to room temperature in glass petri plates. Four sterilized filter paper disc soaked into the different plant extracts of different concentrations were placed on each plate using a sterile forceps. The plates were incubated at 37^0C for 24hrs. After incubation, the zones of inhibition were observed and recorded.

Phytochemical screening of plant extract

Test for Alkaloids (Sofowora, 1984)

About 2g of well ground plant materials was put in a test tube and treated with 1% hydrochloric acid 10ml for 30minutes in a water reagent. Iml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitate with either of these reagents was reagent. Turbidity or precipitate with either of these reagents was taken as presence of alkaloids. Filtration was carried out using a Whatman filter paper.

Test for Saponins (Sofowora, 1984)

About 0.5g of plant extract was shaken with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins. In order to remove the false positive results, the blood haemolysis test was performed on these extracts that frothed in water.

Test for tannins (Sofowora, 1984)

About 5g of plant extract was sterilized with 10ml of distilled water, filtered with Whatman filter paper and ferric chloride reagent was added to the filtrate. Blackish blue precipitate indicates the presence of hydrolysable tannin while blackish green precipitate indicates the presence of condensed tannins.

Test for flavonoids (Cuilel, 1982)

The alcoholic extracts of the plant was added to a few pieces of magnesium metal, conc.HCl was added. The formation of orange, red, crimson was taken as evidence for the presence of flavonoids

Test for glucides (Cuilel, 1982)

The aqueous extract of the plant was evaporated to dryness. Then 2 drops of conc.sulphuric acid was added to it and allowed to stand for 3-5 minutes. The 3 drops of molisch reagent was added to it. The presence of red colour denotes the presence of glycides

Test for cardiac glycoside (Sofowora, 1984)

About 0.5g of the plant extract was dissolved in 2ml of chloroform. Sulphoric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of aglycone portion of the cardiac glycoside.

Test for Anthranoids

Two grams of plant extract was boiled with 5ml potassium hydroxide. The solution was filtered through glass wool. The filtrate was treated with acetic acid and the resulting solution was mixed with toluene. The upper layer was transferred to another test tube and potassium hydroxide added. The presence of red colour indicates the presence of anthranoids.

Test for anthraquinones (Trease & Evans, 1978)

Two grams of plant extract was shakened with 10ml benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free anthraquinones.

Test for phlobatannins (Sofowora, 1984)

About 2ml of plant extract was boiled with 1% aqueous hydrochloric acid. The deposition of red precipitate was taken as evidence for the presence of phlobatannins.

Test for reducing compounds, mucilages, hydroxymethyl anthraquinones were determined in the plant extract using the method of Cuilei, 1982

Estimation of toxic constituent

Estimation of Hydrocyanate acid (A.O.A.C 1975)

Ten grams of plant sample was allowed to soak in 300ml of water for 4 hours for liberation of cyanide. The liberated cyanide was steam distilled into 20ml, 2.5 (w/v) NaOH. 8ml of 6N NH_4OH added to the distillate before titrating with 0.02N $AgNO_3$ to a faint and permanent turbidity.

Determination of Phytate acid (Maccance and Widdowson, 1953)

Two grams of plant sample was extracted with 0.5N HCl, $FeCl_3$ was used to precipitate the phytic acid as ferric phytate and NaOH solution used to convert the precipitate into sodium phytate then digested with acid mix containing equal portions of concentrated H_2SO_4 and $HClO_4$. The liberated phosphorus was qualitated colorimetrically at 620mm after colour development with molybdate solution

Determination of Oxalate

About 5g of the sample was extracted with dilute HCl. The Oxalic acid in the extract was precipitated with calcium chloride as calcium salts. The precipitated oxalate was washed with 25% H_2SO_4 dissolved in heated water before liberating with $KMnO_4$.

Determination of tannin

Ten grams of sample was allowed to soak in 50ml of methanol for 24 hours, with liberation of an extract of tannin. 5ml of fresh vanillin-HCl were added to 1ml portion of this extract and allowed to develop colour in 20 minutes. The absorbance was later measured at 500mm against a reagent blank using spectronic 20.

Proximate composition

The plant sample was analysed for proximate constituents was determined by the standard method laid down by the Association of Official Analytic chemists (A.O.A.C 1975)

Results

Proximate composition of *Aspilia africana*

The proximate compositions of the plant *Aspilia africana* are shown in Table 1, the moisture content is given in terms of wet weight while the other components are given in terms of dry weight. The percentage moisture content was 70% crude protein content was 3.31%, crude lipid (ether extract) content 0.0374%, crude fibre content 7.1% ash content 11.8%, while the carbohydrate content 78.15%.

Toxicant composition

The results of the toxicant composition of *Aspilia africana* (Table3). It was observed that the hydrocyanic acids contents was 3.6mg%. The tannin content was 4.57mg, oxalate content was 9.14 and the phytate content was 1.36mg.

Elemental composition of *Aspilia africana*

The results of the elemental composition of *A.africana* are shown in Table 2. It was observed that the calcium content was 0.247%, Magnesium0.127%, Potassium 0.14%, Sodium 0.118%, Manganese 0.023%, Iron 0.028%, Copper 0.001%, Lead 0.012%, Cobalt 0.001%, Zinc 0.011%, and Phosphorus 0.007%.

Phytochemical components

Table 4 shows the results of the phytochemical components of the aqueous, ethanol, soxhlet, methanol, chloroform extracts. The results reveal that the aqueous, ethanol, soxhlets, methanol and chloroform extracts all contains saponins, alkaloids, cardiac glycosides. Aqueous, ethanol, soxhlet contain hydrolysable tannins. Only ethanol, methanol and chloroform extracts contained condensed tannins. The chloroform extract contained flavonoids and soxhlets extraction contained glucosides. Soxhlet extraction, methanol and chloroform extract contained anthranoid. The aqueous extracts contained polyphenols. Reducing compounds were found only in aqueous and ethanol extracts. Mucilage was contained in ethanol, soxhlet, methanol extracts only chloroform extract contained phlobatannins. Aqueous and soxhlet extraction contained hydroxymethyl anthroquinones.

Antimicrobial test of the various extracts

The results of the antimicrobial tests of the crude water, soxhlet, methanol, chloroform and ethanol extracts of *A. africana* on microorganisms, bacteria and fungi.

Table 5 shows that there was inhibition of growth of *streptococcus pyogenese* and *Bacillus subtilis* at the various concentrations of the crude water at 10%, 20%, 30% and 40%. The following organisms were resistant to the extracts (crude water), *Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Mucor spp.* Table 9, indicates that there was inhibition of *S. pyogenese*, *Proteus vulgaris*, *Staphylococcus aureus, Streptococcus faecalis* at various concentrations of 10%, 20% and 30% and *Klebsiella pneumonia* only at 40% by the soxhlet extraction. *Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Candida albicans, Mucor* proved resistant. *Klebsiella pneumonia* shows resistant to 10%, 20%, 30% concentration. Table 7 the chloroform extract inhibited the growth of *S. pyogenese and S. faecalis*. There was inhibition of *Mucor and Staph aureus* at 10% and 20% of the extract concentration. *Proteus vulgaris, E.coli and Kleb pneumonia* showed the largest zones of inhibition ranging from 26±0.2 to 27.6±0.2, 28±0.14 to 29±0.26, 26±0.2 to 27±0.29 respectively. *Mucor and Staph aureus* were inhibited but showed little sensitivity of 26±0.01 at concentrations of 30% and 40%. Table 8 shows zones of inhibition by ethanol extract. There was no zone of inhibition to the growth of *Proteus vulgaris*. There was slight inhibition to *Candida albicans* only at 40% concentration of the extract. *Bacillus subtilis* showed inhibition of 25±0.1 at 30% and 40% of the extract. Very small inhibition of 25±0.05 was observed on *E.coli*. The largest scale of inhibition was recorded by *Strep faecalis* at a range of 26±0.1 to 25.5±0.28 inhibition occurred also to *S. pyogenese, Mucor, Staph aureus, Kleb pneumonia*. The control shows no zone of inhibition. There was profused growth in all the plates.

Table 1 Proximate composition of the *A.africana*

Composition	Percentage content
Crude protein	3.31±0.12
Moisture content	70.0±0.01
Ash content	11.8±0.02
Fibre content	7.10±0.01
Fat content	0.37±0.11
Carbohydrate	78.15±0.10

Data were express in mean and standard error

Table 2 Elemental composition of *Aspilia Africana*

Elements	Content
Calcium (Ca)	0.247
Magnesium (Mg)	0.127
Potassium (K)	0.142
Sodium (Na)	0.118
Manganese (Mn)	0.023
Iron (Fe)	0.028
Copper (Cu)	0.001
Lead (Pb)	0.012
Cobalt (Co)	0.001
Zinc (Zn)	0.011
Phosphorus (P)	0.007

Table 3 Toxicant composition of *Aspilia africana* (mg)

Toxicant	Content
Hydrocyanic acid	3.60±0.10
Tannic acid	4.57±0.01
Oxalic acid	9.41±0.03
Phytic acid	1.36±0.02

Data were express in mean and standard error

Table 4 Phytochemical composition of various extracts of *Aspilia Africana*

Parameters	Aqueous extracts	Ethanol extract	Soxhlet extracts	Chloroform extracts	Methanol extract
Saponins	+	+	+	+	+
Condensed tannins	-	+	-	+	+
Hydrolysable tannins	+	+	+	-	-
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	-	-
Glucides	-	-	+	-	-
Cardiac Glycosides	+	+	+	+	+
Anthranoids	-	-	+	+	+
Polyphenols	+	-	-	-	-
Reducing compounds	+	+	-	-	-
Muscillages	-	+	+	-	+
Phlobatannins hydroxymethyl	-	-	-	+	-
Anthraquinones	-	+	+	-	+

Table 5 Antimicrobial tests (zones of inhibition) in mm of the crude water extract at various concentrations

Microorganisms	10%	20%	30%	40%
Strep pyogenese	28.0±0.21	26.9±0.29	27.2±0.12	26.8±0.32
Proteus vulgaris	-	-	-	-
Bacillus substilis	26.8±0.23	26.5±0.12	26.5±0.22	26.5±0.2
Stap aureus	-	-	-	-
Strep faecalis	-	-	-	-
E.coli	-	-	-	-
Klebsiella pneumonia	-	-	-	-
P. aeruginosa	-	-	-	-
Candida albicans	-	-	-	-

Data were express in mean and standard error.

Table 6 Antimicrobial test (zones of inhibition) in mm of the methanol extract at various concentration

Microorganisms	10%	20%	30%	40%
Strep pyogenese	26.63±0.14	25.75±0.15	27.63±0.14	26.37±0.07
Proteus vulgaris	-	-	-	-
Bacillus substilis	27.4±0.07	25.75±0.09	27.0±0.24	29.6±0.25
Stap aureus	27.35±0.12	26.5±0.17	28.25±0.08	27.0±0.27
Strep faecalis	26.5±0.17	27.12±0.03	27.0±0.34	28.5±0.17
E.coli	26.25±0.12	28.75±0.43	35.75±0.09	27.09±0.21
Klebsiella pneumonia	27.63±0.22	28.25±0.14	28.6±0.14	26.6±0.17
P. aeruginosa	-	-	-	-
Candida albicans	27.25±0.3	25.75±0.15	27.62±0.14	27.4±0.06

Data were express in mean and standard error.

Table 7 Antimicrobial test (zones of inhibition) in mm of the soxhlet extracts at various concentration

Microorganisms	10%	20%	30%	40%
Strep pyogenese	26.0±0.27	26.7±0.06	26.0±0.27	25.0±0.33
Proteus vulgaris	-	-	-	-
Bacillus substilis	26.0±0.17	27.0±0.24	26.6±0.48	26.0±0.17
Stap aureus	-	-	-	-
Strep faecalis	-	-	-	-
E.coli	-	-	-	-
Klebsiella pneumonia	-	-	-	-
P. aeruginosa	29.0±0.25	26.0±0.12	20.5±0.31	30.5±0.3
Candida albicans	27.0±0.12	27.8±0.22	28.0±0.43	27.5±0.24

Data were express in mean and standard error.

Table 8 Antimicrobial test (zones of inhibition) in mm of the ethanol extract at various concentration

Microorganisms	10%	20%	30%	40%
Strep pyogenese	25.5±0.09	25.5±0.09	26.0±0.08	27.0±0.02
Proteus vulgaris	-	-	-	-
Bacillus substilis	-	-	-	-
Stap aureus	26.5±0.28	26.0±0.10	26.0±0.03	26.5±0.28
Strep faecalis	26.0±0.17	26.8±0.03	25.5±0.28	25.5±0.29
E.coli	27.4±0.07	26.5±0.17	27.0±0.34	27.09±0.06
Klebsiella pneumonia	-	-	-	-
P. aeruginosa	-	-	-	-
Candida albicans	27.63±0.22	28.25±0.14	28.63±0.11	26.6±0.17

Data were express in mean and standard error.

Table 9 Antimicrobial test (zones of inhibition) in mm of the chloroform extracts at various concentration

Microorganisms	10%	20%	30%	40%
Strep pyogenese	-	-	-	-
Proteus vulgaris	26.05±0.21	27.2±0.25	27.6±0.22	27.5±0.23
Bacillus substilis	27.0±0.32	27.0±0.22	28.5±0.23	28.0±0.12
Stap aureus	-	-	-	-
Strep faecalis	-	-	-	-
E.coli	28.0±0.14	28.0±0.14	28.0±0.25	29.0±0.26
Klebsiella pneumonia	26.0±0.20	27.0±0.29	26.5±0.30	26.5±0.26
P. aeruginosa	26.0±0.07	26.5±0.08	26.5±0.17	27.5±0.07
Candida albicans	26.5±0.07	26.0±0.09	26.0±0.01	27.5±0.05

Data were express in mean and standard error.

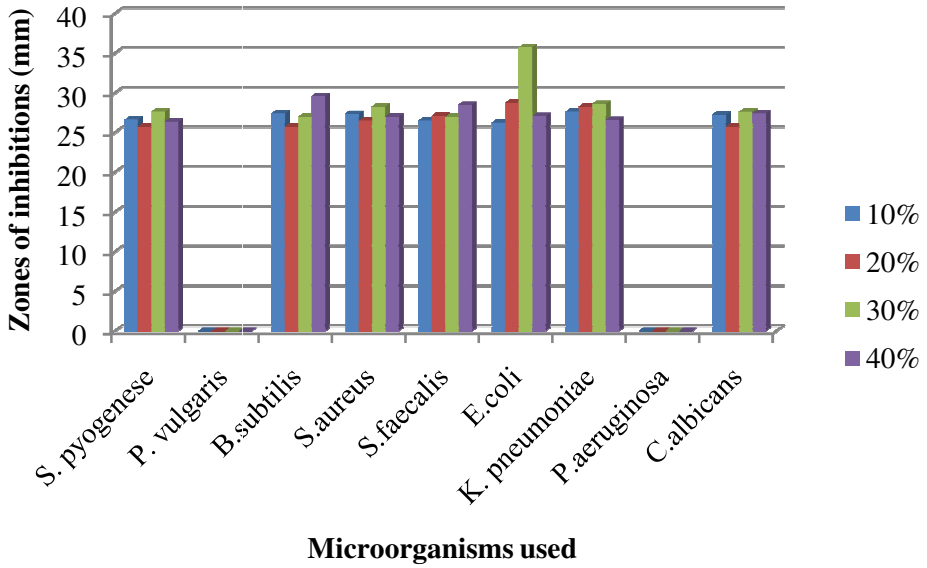

Fig. 1 Zones of inhibition of microorganisms using methanol extract of *Aspilia africana* at different concentration

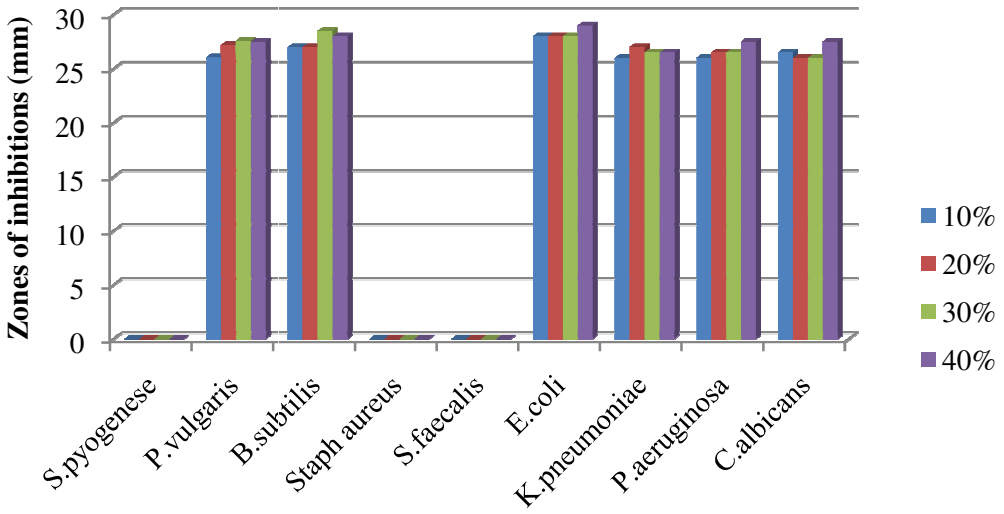

Fig.2: Zones of inhibition of microorganism using chloroform extracts of *Aspilia africana* at different concentrations

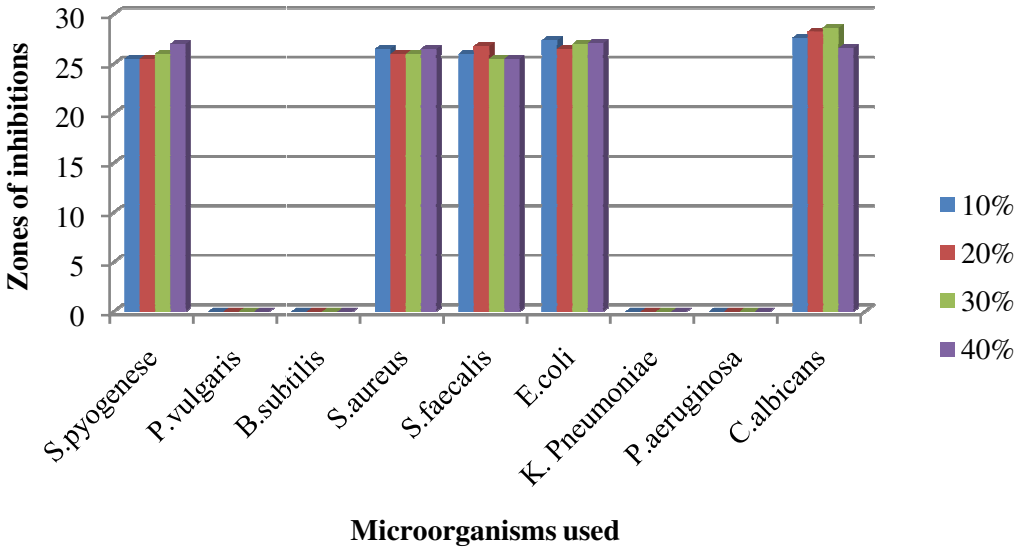

Fig.3: Zones of inhibition of microorganism using ethanol extracts of *Aspilia africana* at different concentrations

Discussion

The leaves of *A. africana* possess constituents capable of arresting wound bleeding, inhibiting the growth of microbial wound contaminants and accelerating wound healing which suggest good potentials for use in wound care. The leaves of *Aspilia africana* was used in determining the chemical and antimicrobial properties of the plant. The proximate composition of the plant shows that the moisture content, crude carbohydrate, fibre content, protein, ash and fat content were high. The oxalic acid, tannic acid and phytic acid were also high in the sample. It has been suggested that if fermentation using various yeast as previously reported by Essien *et al*., 1992, these toxicant might be greatly reduced. Other interesting composition of this plant is the elemental composition. It is interesting that this plant has very low content of heavy toxicant metals such as cobalt, cadium and lead. This indicates that at least the low toxicity of this plant cannot be due totally to toxicant elements. Other elements though not high in concentration are elements useful to the body. Another chemical composition studied was phytochemical components; the presence of these compounds is an indication that such a plant might contain bioactive components that may exhibit inhibitory activity on microorganisms (Ebana *et al*., 1986)

The aqueous, ethanol, soxhlet, chloroform and methanol extracts of *Aspilia africana* all have saponins, alkaloids and cardiac glycosides. Phlobatannins was only present in the chloroform extracts. Flavonoids were absent in all the extracts. The hydrolysable tannins were present only in the aqueous, ethanol, and soxhlet extracts. Condensed tannins were present only in the ethanol, chloroform, methanol extracts. Hans (1952) also attributed the antimicrobial and haemostatic activities of plant extracts on fresh wounds to tannins. Glucides was present only in soxhlet extracts. Anthronoids were absent in the aqueous and ethanol extracts. Polyphenols was present in the aqueous extracts reducing compounds. Hydroxymethyl anthrquinones were present in aqueous and soxhlet extracts. The high antimicrobial activities observed in the plant could be attributed to the presence of tannins and alkaloid which as the potentials in wound healing due to the ability of tannin to bind to proteins of exposed tissues thus, precipitating the protein.

Proteus vulgaris was resistant to crude water, methanol, ethanol extracts. Its growth was inhibited by soxhlet and chloroform extracts. Such an inhibition is speculated to have been the saponins, alkaloids, cardiac glycosides and anthranoids. *Bacillus subtilis* was inhibited by crude water, methanol, chloroform and ethanol extracts. Probably, it is due to the presence of saponin, alkaloids, cardiac glycosides. *Staph aureus* was inhibited by methanolic, ethanolic and soxhlet extracts. The components probably responsible should be hydroxymethyl anthraquinones, mucillages in addition to saponins, alkaloids, cardiac glycosides. However, in this study was observed that the methanol, ethanol and chloroform *of Aspilia africana* exhibited a higher zones of inhibition on microbial proliferation (Fig. 1-3).

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