

Screening of Anthraquinones and Assessment of Antimicrobial Activities of Ethanol Extracts of *Adansoniadigitata*L., Sudan

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Abstract: The aim of the study was to investigate anthraquinones and to determine the antimicrobial activity of different extracts of *Adansoniadigitata* L., a plant used by Sudanese communities to treat various ailments such as diarrhea and microbial infections. The anthraquinones were screened qualitatively. The presences of anthraquinones were detected in ethanol extracts along with acetone and ethyl acetate fractions of both fruits fibers and fruits cover of *A. digitata*. The fruits fibers and fruits cover extracts were screened for antimicrobial activity against Gram- positive (*Bacillus subtilis* and *Staphylococcus aureus*), and Gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*), and two fungi (*Candida albicans* and *Aspergillus niger*) using agar well diffusion method. Results showed that the fruits covers extract was exerted highest activity on bacterial agents tested compared to fruits fibers extract, and fruits fibers extract was the most effective against fungi (*A. niger*), while both extracts were not active against *C. albicans*. Fruits cover extract at concentration of 100 µg / ml showed 25 mm diameter zone of inhibition against *P. vulgaris*. This was followed by 23, 22 and 21 mm zone of inhibition against *B. subtilis*, *K. pneumoniae* and *S. aureus*. TLC analysis revealed the presence of two anthraquinones compounds in the acetone extract of the fruits cover.

Keywords: *Adansoniadigitata*, Antibacterial, Antifungal, Anthraquinones, Sudan

1. Introduction

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in International trade [1]. The medicinal value of plants lies in the presence of chemical substances found as secondary plant metabolites that definitely produce a physiological action on the human body. The interest in using phytochemicals for the treatment of microbial infections increased in the late 1990's with the increased inefficacy of conventional antibiotics, due in part to their often excessive and in appropriate use in mammalian infections [2]. The most important of these bioactive compounds in plants are alkaloids, anthraquinones, flavonoids, saponins, tannins and other chemical compounds [3],[4].

The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [5]. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases [6].

Thus, the aim of this study was to evaluate the activity of the fruits covers and fruits fiber extracts as well as to detect the presence of anthraquinones in *Adansoniadigitata* L. a plant traditionally used for medicinal purposes in Sudan.

2. Review of Literature

2.1. *Adansoniadigitata* L.

A. digitata L. (Malvaceae) (Fig. 1), the African baobab, is a tree widespread throughout the hot regions of tropical Africa [7]. It is a deciduous, massive and majestic tree up to 25 m high, which may live for hundreds of years [8]. In Sudan, the baobab tree is called *Tebaldi*, and it is most frequently found on sandy soils and by seasonal streams in short grass savannas. It forms belts throughout Central Sudan, Kordofan, Darfur and Blue Nile regions [9].



Figure 1: *A. digitata* (baobab) tree

2.1.1 Medicinal and folkloric uses of *A. digitata*

Baobab has a long history of traditional uses as a treatment against fevers, dysentery, and bleeding wounds, and it has a long history of nutritional and medical use in Africa [10]. Baobab fruits and leaves are used in folk medicine by communities of North Kordofan, Sudan against dysentery,

stomach pains, diarrhea, fever and the infusion of the fruits covers for kidney stones [11]. The aqueous extract of baobab fruit pulp exhibited significant hepatoprotective activity [12]. Ramadan *et al.*, 1994 reported that the analgesic and antipyretic activities of baobab may be due to the presence of sterols, saponins and triterpenes [13]. The fruit can be used for the treatment of fever, diarrhea, dysentery, hemoptysis and small pox [14].

Previous phytochemical studies of various parts of baobab (fruit pulp, bark, seed oil, leaves, roots) have resulted in the identification and isolation of various phytochemicals including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids [15].

2.1.2 Antimicrobial activity of *A. digitata*

Masola *et al.* (2009) found that the aqueous and ethanolic extracts of the root and stem bark of *A. digitata* was active against Gram-positive bacteria, Gram-negative bacteria and yeast. The results indicated that these extracts inhibited the growth of the microorganisms tested with MIC values ranging from 1.5 to 6 mg/ml. The antibacterial activity of the plant could be attributed to the presence of tannins, terpenoids and saponins in the stem and root bark [16]. This result was in agreement with the study carried out by Ananiet *al.* (2000) and Yagoub (2008) who investigated the antibacterial activity of a solvent extract of *A. digitata* against *E. coli* isolated from urine and water [17], [18]. The results clearly indicated that the solvent extract inhibited bacterial growth with the inhibition zone ranging from 20 to 30 mm depending on the concentration at which the sample was tested [18].

2.2 Anthraquinones

In plants, anthraquinones are found in a wide range of species, especially in the plant families Rubiaceae, Polygonaceae, Rhamnaceae, Fabaceae, and Xanthorrhoeaceae [19]- [22]. Anthraquinone compounds and plants that contain them are widely used as mild laxatives [23], [24]. Besides their purgative properties, anthraquinones possess antibacterial, antiviral [25], antifungal [26], antioxidant [27] and anticancer [28] properties.

3. Materials and Methods

All chemicals, solvents and reagents used were of analytical grade. Most of the chemicals used were supplied by Fisher Scientific (Springfield, NJ), British Drug Houses (England) and Sigma® (Germany), and were of the purest grade available.

3.1. Plant Materials

The plant materials, fruits fibers and fruits covers (Fig. 2) of *A. digitata* were collected from the area around El Obeid city the capital of Northern Kordofan state, Sudan in December 2013 and authenticated by the researchers at National Research Center, Khartoum, Sudan. Plant materials were dried under shade and pulverized into powdered forms. They were then stored in air tight bottles and were used for all the extraction process.



a



b

Figure 2: (a) *A. digitata* (baobab) fruit; (b) fruit cover and fibers

3.2 Extraction

3.2.1. Maceration extraction

Hundred grams of powdered plant material was extracted three times with ethanol during three days at room temperature. The extract was filtered and the solvent was removed under reduced pressure, then air dried. Same process was done for making extracts of both fruits fibers and fruits cover.

3.2.2. Soxhlet extraction

Dried and powdered fruits fibers (20 g) of *A. digitata* was successively extracted in a Soxhlet apparatus, with hexane followed by ethyl acetate, acetone, and finally methanol with 200 ml of each solvent for 6 hours. The extracts were filtered before drying using Whatman filter paper No. 2 on a Büchner funnel and the solvent removed by vacuum distillation in a Büchi rotary evaporator (BÜCHI Rota-vapor R-200) at 60 °C, care being taken to decrease the temperature to 40°C for the final drying. For quantitative determination, the extracts were placed in pre-weighed flasks before drying. The dried extracts were kept in a refrigerator till used. This process was repeated for extraction of fruits cover of *A. digitata*.

3.3 Test for anthraquinones

The extract (0.5 g) was shaken with an aliquot of 10 mL of benzene. The mixture was filtered and an aliquot 5 mL of

10% ammonia solution was added to the filtrate and the final mixture was shaken and the two layers allowed separating. The presence of anthraquinones was indicated by pink or red coloration of the lower alkaline layer [29], [3].

3.4 Antimicrobial activities

3.4.1 Microorganisms used

The bacteria used were *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Proteus vulgaris* (ATCC 6380) and *Klebsiella pneumoniae* (ATCC 53657). The fungi used were *Candida albicans* (ATCC 7596), and *Aspergillus niger* (ATCC 9763).

3.4.2 Antimicrobial activity test

The agar-well diffusion method as described by Irobi et al., (1996) [30], with modifications was adopted to assess the antibacterial and antifungal activities of the crude extracts. The bacterial cultures were inoculated onto nutrient agar medium. A sterile cork borer was used to make a well (6 mm in diameter) on the Nutrient Agar plates. 100 µl of each extract at concentrations of 100 µg/ml, were applied separately in each of the wells in the culture plates previously seeded with the test organisms. Plates were incubated at 37 °C for 24 h. Controls were set up in parallel using the solvents that were used to reconstitute the extract. Antibacterial activities were evaluated by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract, three replicate trials were conducted against each organism.

The same method as for bacteria was adopted for antifungal activity. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 °C for one day for *C. albicans* and two days for *A. niger*.

Extracts causing a zone of inhibition of > 20 mm were considered highly active and those having a zone of inhibition of < 20 mm were considered moderately active.

3.5 Thin layer chromatography

Acetone and ethyl acetate extracts obtained by Soxhlet extraction for both fruits cover and fruits fibers were subjected to thin layer chromatography (TLC) for the separation of polar and non-polar compounds. The solvent system used as mobile phase was toluene: ethyl acetate: formic acid (4:5:1, v/v). The plates were sprayed with natural product reagent and visualized under ultraviolet light (365 nm). Identification was done on the basis of color and Rf value under UV light at 365 nm.

4. Results and Discussion

4.1 Test for anthraquinones

The ethanol extracts obtained by maceration method along with hexane, ethyl acetate, acetone and methanol extracts obtained from Soxhlet extraction for both fruits fibers and fruits cover were tested for the presence of anthraquinones. In the screening process the two ethanol extracts along with

ethyl acetate and acetone extracts (of both fruits fibers and fruits cover) were given positive results (pink to red coloration), while hexane and methanol extracts gave negative results. For the best of our knowledge this is the first study indicating the presence of anthraquinones in *A. digitata*. The presence of anthraquinones in the fruits fibers and fruits cover extracts may be responsible for the antimicrobial activity of the plant extracts.

4.2 Assessment of antimicrobial activities

Preliminary screening of the antimicrobial activity in vitro of fruits fibers and fruits cover ethanol extracts was studied against eight pathogen microorganisms. The results showed variation in the antimicrobial properties of plant extracts (Table 1 and Fig. 3-10). The fruits cover extract exerted highest activity on bacterial agents tested compared to fruits fibers extract. The fruits fibers extract at the concentration of 100 µg / ml showed 25 mm diameter zone of inhibition against *P. vulgaris*. This was followed by 23, 22 and 21 mm zone of inhibition against *B. subtilis*, *K. pneumoniae* and *S. aureus*. The results showed that the fruits fibers extract was more effective than fruits cover extract against fungi (*A. niger*), while both extracts were not active against *C. albicans*. However, the fact that the fruits cover extract was active against both gram-negative and gram-positive bacteria may provide scientific bases for the local usage of this plant part in the treatment of various ailments, especially dysentery and diarrhea.

Table 1: Antimicrobial activity of extracts

Microorganisms	Inhibition zone (mm)	
	Ethanol extracts	
	Fruits cover	Fruits fibers
<i>E. coli</i>	20	19
<i>P. aeruginosa</i>	20	18
<i>K. pneumoniae</i>	22	19
<i>P. vulgaris</i>	25	-
<i>B. subtilis</i>	23	-
<i>S. aureus</i>	21	18
<i>C. albicans</i>	-	-
<i>A. niger</i>	20	22

* >20mm = highly active; 15 – 20 mm = moderately active; - = Negative

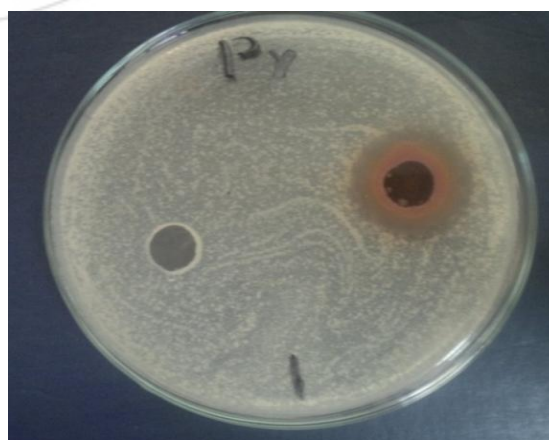


Figure 3: Inhibition zone of the ethanol extracts of fruits cover against *P. vulgaris*

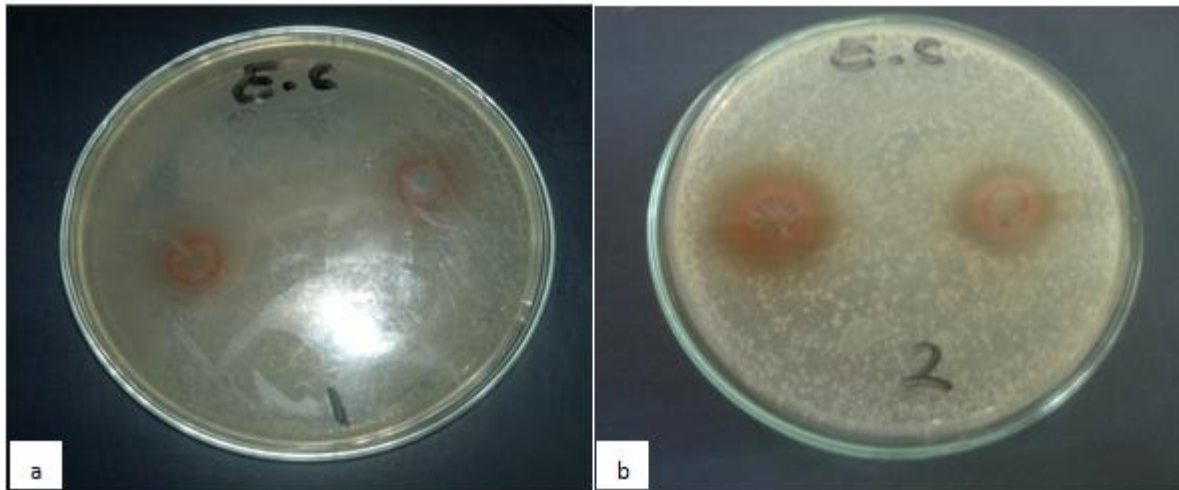


Figure 4: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *E. coli*

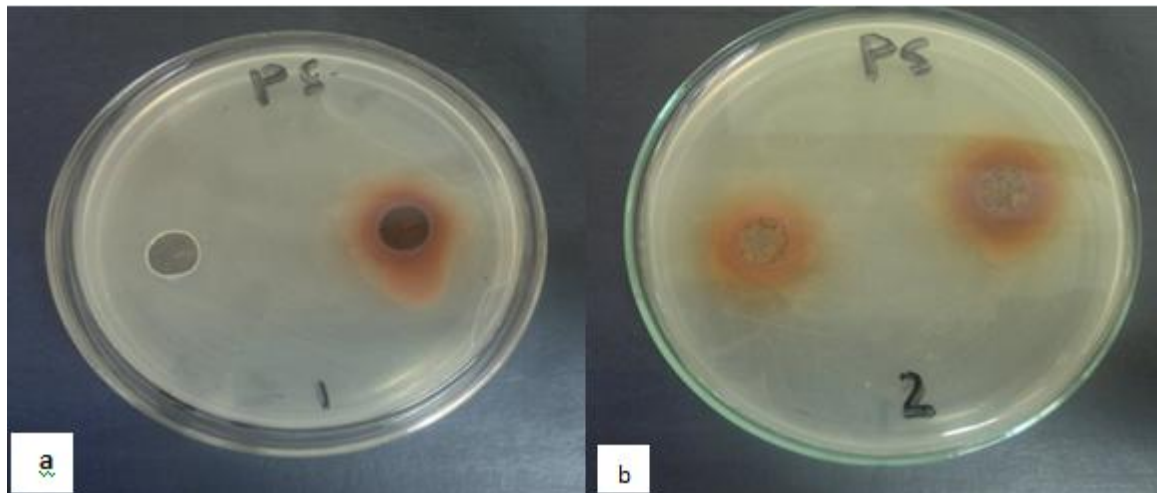


Figure 5: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *P. aeruginosa*

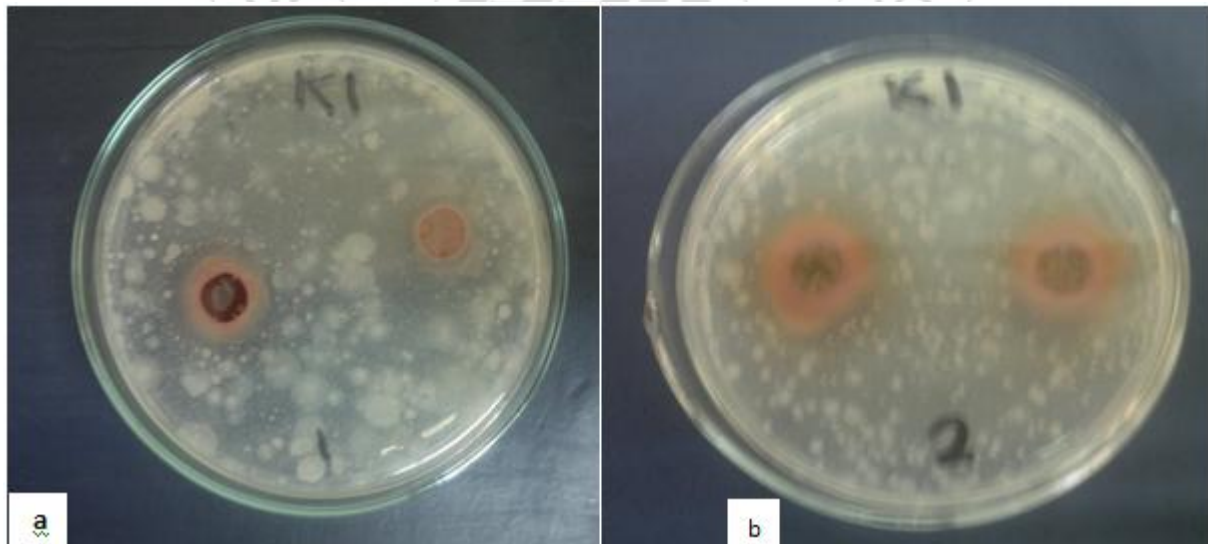


Figure 6: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *K. pneumoniae*

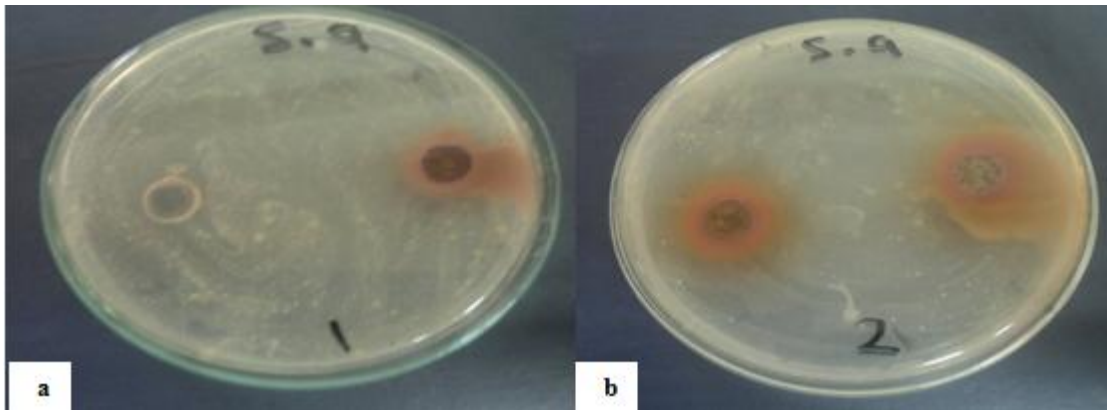


Figure 7: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *S. aureus*

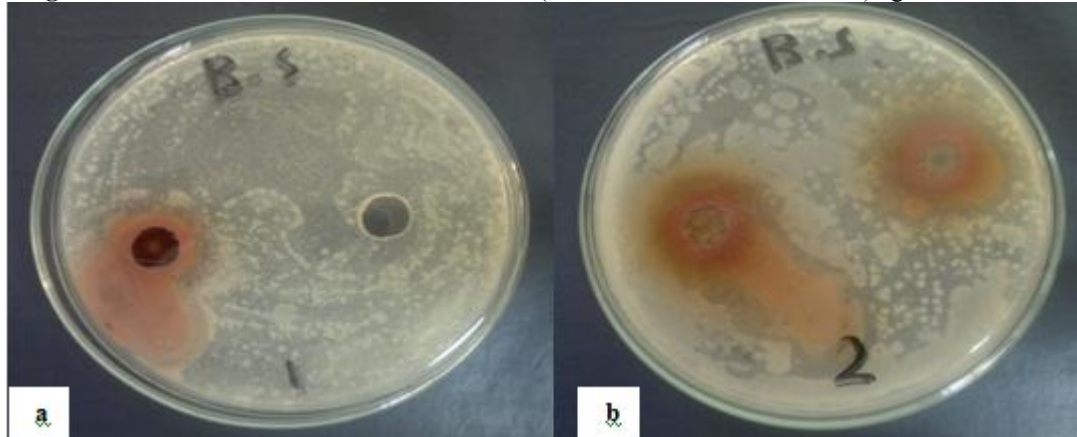


Figure 8: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *B. subtilis*

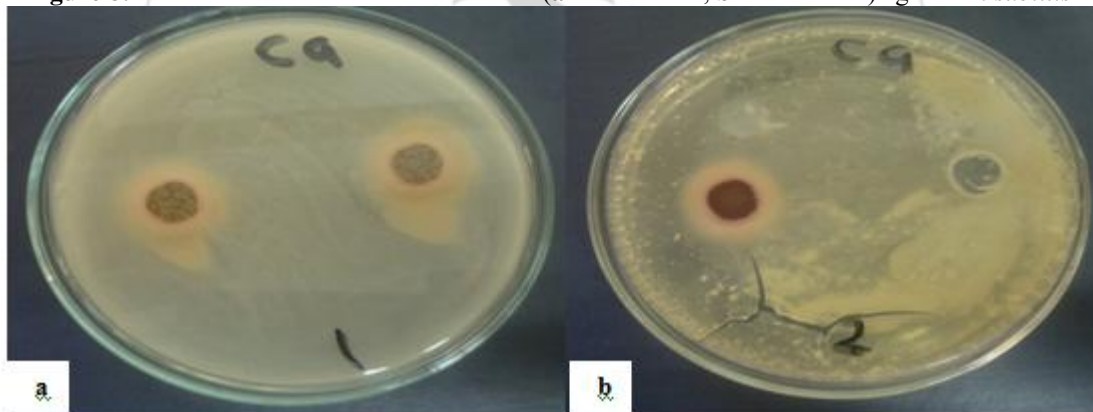


Figure 9: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *C. albicans*

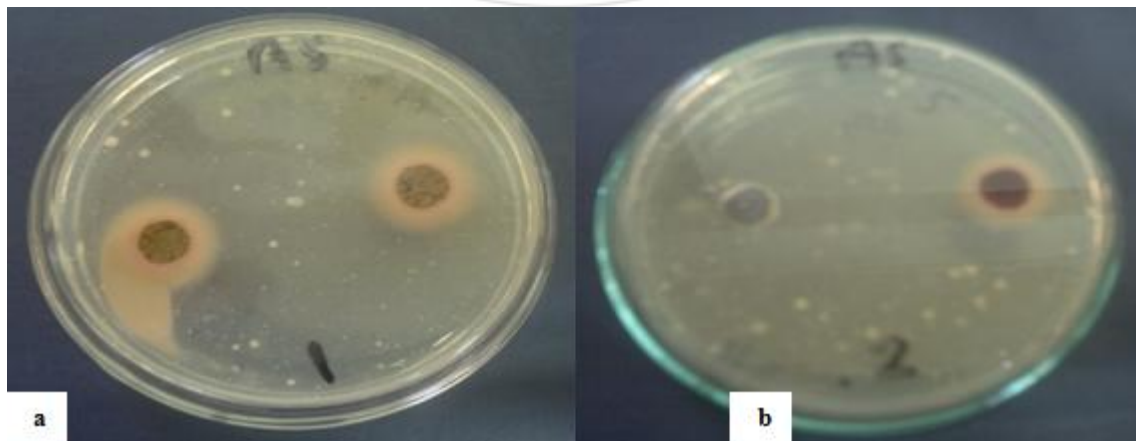


Figure 10: Inhibition zone of the ethanol extracts (a: fruits fibers; b: fruits cover) against *A. niger*

4.3 TLC analysis of acetone and ethyl acetate extracts of fruits cover

Acetone and ethyl acetate extracts obtained by Soxhlet extraction for both fruits cover and fruits fibers were subjected to thin layer chromatography performed on a precoated aluminium plate of silica gel 60F₂₅₄ (10 x 20 cm) using toluene: ethyl acetate: formic acid (4:5:1) as the mobile phase. After removing the plate from the chamber, the plate was dried using an air dryer and spray with natural product reagent then visualized under UV 365. Anthraquinones show yellow spots [31]. The R_f values and colors of the separated compounds are shown in Table (2). UV active compounds, component-1 (retardation factor or R_f value = 0.35 and coloration Brown/Yellow, the compound would be anthraquinone), and component-2 (R_f = 0.55 and coloration Yellow/ Yellow, the compound would be anthraquinone) were observed in the acetone extract of fruits cover.

Table 2: TLC of acetone extract of the fruits cover

Components	R _f value	Color with spray reagent	Color under UV 365nm
1	0.35	Brown	Yellow
2	0.55	Yellow	Yellow

- Mobile phase: toluene: ethyl acetate: formic acid (4:5:1)
- Stationary phase: silica gel 60F₂₅₄
- Spray reagent: natural product reagent (NPR)

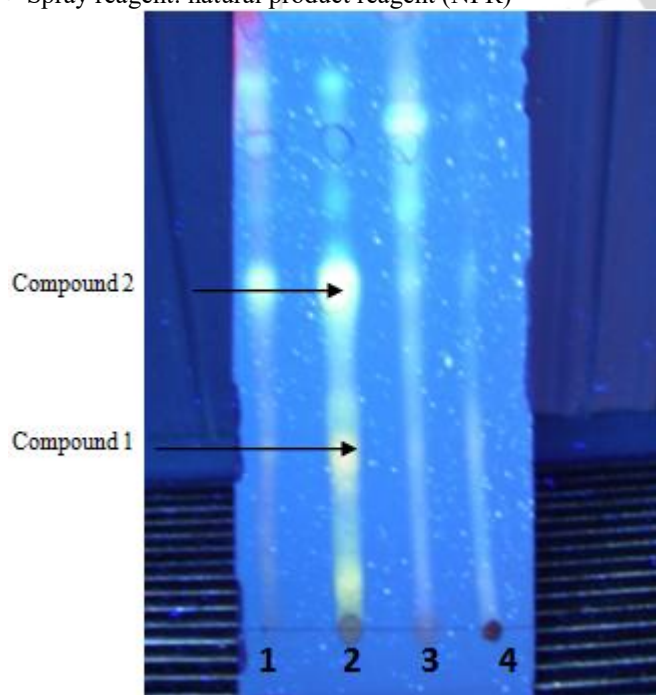


Figure 11: TLC of *A. digitata* extracts sprayed with NPR and detected under UV 365 nm (1 = ethyl acetate extract (fruits cover); 2 = acetone extract (fruits cover); 3 = ethyl acetate extract (fruits fibers); 4 = acetone extract (fruits fibers))

5. Conclusion

In this study the ethanol extracts of fruits cover and fruits fibers of *A. digitata* were screened for antimicrobial activity using agar-well diffusion method. The extracts were also screened for the presence of anthraquinones. The fruits

covers extract exerted highest activity on bacterial agents tested compared to fruits fibers extract and fruits fibers extract was the most effective against fungi (*A. niger*), while both extracts were not active against *C. albicans*. The fruits cover extract showed 25 mm diameter zone of inhibition against *P. vulgaris*. Phytochemical screening of anthraquinones showed positive results for both extracts of *A. digitata*. TLC analysis revealed the presence of two anthraquinones in acetone fraction of the fruits cover. The antimicrobial activity may attribute to anthraquinones. However, the activity of *A. digitata* different extracts against both gram-negative and gram-positive bacteria strains tested may provide scientific bases for the local usage of the plant in the treatment of various ailments, especially dysentery and diarrhea.

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