*Original*

# Preliminary Phytochemical Screening, Antimicrobial and Antioxidant Activities of *Xylopia aethiopica* (Dunal) A. Rich Fruits

Elshiekh Y.H[*1], Alnoor G.H[2], Hussein S.E[2], Abdalrhman M.I[2], Rahmtalla H.A[2]

[1.] Department of Biology &Technology, College of Applied and Industrial Sciences, University of Bahri, Khartoum, Sudan.

[2.] Department of Pharmacognosy, Faculty of Pharmacy, Omdurman Islamic University, Khartoum, Sudan

***corresponding author:** Dr. Yasmin Hassan Elshiekh, Assistant professor, University of Bahri, Khartoum, Sudan. Email: yasmin_hassan13@yahoo.com

Received: 28 May2022

Accepted: 18 June 2022

## Abstract

**Background:** This current study was carried out to investigate possible antibacterial; antioxidant activities and phytochemical screening of Petroleum ether, ethyl acetate and 70% ethanol extract of *Xylopia aethiopica*.

**Materials & Methods:** Phytochemical analysis of the crude extract was performed to detect the presence of different kinds of phytoconstituents. The antibacterial activity was investigated against five standard microbial strains: four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) and one standard strain of fungi (*Candida albicans*) by using the disc diffusion method.

**Results:** All extracts showed high, moderate or weak antimicrobial activity against all the strains used. Antioxidant activity showed that, the ethanol extract was the most active extract (60±0.07) in comparison to the other extracts. **Conclusion:** Phytochemical screening showed the presence and absence of the secondary metabolites among the entire sample tested.

**Keywords:**

*Xylopia aethiopica*, Annonaceae, Antimicrobial, Antioxidant, Phytoconstituents, .

ISSN: 2948-300X (print) 2948-3018 (0nline)

## Introduction

*Xylopia aethiopica* (Annonaceae) is widely distributed in the West African rainforest from Senegal to Sudan in Eastern Africa, reaching Angola in Southern Africa (1, 2*). X. aethiopica* is a medicinal plant of great repute in West Africa, and contains a variety of complex chemical compounds (3). These active ingredients are extracted in various forms such as crude aqueous, organic extracts or in the form of essential oils. The medical importance of X. *aethiopica* has been extensively reported previously [4-8]. Several reports on the antimicrobial activity and composition of the essential oil of *Xylopia aethiopica* fruit have been investigated. The essential oil as well as the crud extracts (both alcoholic and aqueous) of the plant has been shown to have antimicrobial property against bacteria, and *Candida albicans* [8-13]. Almost every part of the plant is used as a traditional medicine for managing various ailments including skin infections, candidiasis, dyspepsia, cough and fever (1, 2, 14). In Congo, decoction of the *X. aethiopica* fruit is used in the treatment of bronchitis, asthma, stomach-aches and dysenteric conditions. Mixture of *X. aethiopica* with salt is used as a cure for constipation. Its decoction is used in Gabon against rheumatism (2). It is taken to encourage fertility and to ease child birth. When crushed, *X. aethiopica* is used to treat headache and neuralgia. An extract of the seeds is also used as vermifuge for round warms (15). On the other hand, many pharmacological studies of *X. aethiopica* different extracts were carried out on rats [16-18].

Phytochemical screening of the fruit of *X. aethiopica* confirmed the presence of saponin, saponin glycoside, tannin, balsam, cardiac glycoside and volatile oil (19). Spectrophotometric analysis for trace metals (such as Mg, Zn, Cu, Ni and Fe), Phosphorus and Sulphur showed that *X. aethiopica* fruit contained Mg (0.370 + 0.002 mg/100g), Zn (1.020 + 0.001 mg/100g), Cu (0.274 + 0.004 mg/100g), Ni (1.099 + 0.001 mg/100g), Fe (0.690 + 0.002 mg/100g), P (30.62 + 0.02 mg/100g) and S (100.50 + 0.51 mg/100g)[16].

The objective of this research is to detect the presence of secondary metabolites and to investigate the antibacterial and antioxidant activities of *Xylopia aethiopica* fruits.

## Materials and Methods:

### Plant material

The fruit part of *X. aethiopica* was brought from a local market in Omdurman and authenticated by Dr. Yahiya Suleiman taxonomist at herbarium of Medical and Aromatic Plants & Traditional Medicine Research Institute (MAPTRI), Department of botany in the National Center for Research.

### Preparation of the plant material

Plant was cleaned, freed from dust and foreign material, and finally crushed manually.

### Preparation of the crude extracts

A weight of 100 grams of the coarsely powdered sample was successively extracted using different solvents.

### Continuous extraction method

The crushed powdered form of *X. aethiopica* (100g) was thoroughly extracted using Soxhlet apparatus with different organic solvents (Petroleum ether, ethyl acetate and ethanol) in order to increase polarity. Each extract was filtered and evaporated under reduced pressure using Rotary evaporator (20). The percentage of different extract yields were then calculated and tabulated. The different extracts were preserved in the refrigerator till time of use.

### General Phytochemical Screening

Phytochemical Screening for the active constituents was carried out for extracts using the methods described at references [20-23] with some modifications.

Plant was cleaned, freed from dust and foreign material, and finally crushed manually. Then was weighted, the sample after that was ready for the extraction method.

### Test for alkaloids

0.5g of the extract was heated with 5ml of 2NHCl in water bath and stirred for about 10 minutes, then cooled, filtered and divided into two test tubes. In a single test tube a few drops of Mayer's reagent was added and a few drops of Valser's reagent was added to the other tube. A slight turbidity or heavy precipitate in either of the test tubes was tanked as presumptive evidence for the presence of alkaloids.

**Test for flavonoids**

0.5g of the extract was dissolved in 30 ml of 80% ethanol; the filtrate was used for following tests:

A/ 3ml of filtrate was added in a test tube containing 1ml of 1% aluminum chloride solution with methanol, the appearance of yellow color indicates the presence of Flavonoids, Flavones or and chalcone.

B/ 2ml of filtrate 0.5ml of magnesium turnings were added, defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant.

**Test forcoumarins:**

In a test tube, 0.5g of the extract dissolved in 10 ml distilled water with a filter paper attached to it to be saturated with the vapor after a spot of 0.5N KOH was added. Then the filter paper was visualized under UV light, the presence of coumarins was indicated if the spot was found to be absorbed under UV light.

**Test for saponins:**

0.5g of the extract was placed in a clean test tube. 10ml of distilled water was added; the tube stoppered and was vigorously shaken for about 30 seconds. It was later observed for the formation of foam, which persisted for at least an hour, and was taken as an evidence for the presence of saponins.

**Test for triterpenes and sterols:**

0.5g of the extract was dissolved in 10ml of chloroform. 0.5ml acetic anhydride was added to 5ml of the solution and 3 drops of concentrated sulphuric acid was added to the test tube. At the inter phase of the two liquids, the gradual appearance of green, blue, pink to purple color was taken as an evidence of the presence of sterols (green to blue color) and or triterpenes (pink to purple) in the sample.

**Test for tannins:**

0.5g of the extract dissolved in 10ml hot saline and divided in two test tubes. To one test tube, 2-3 drops of ferric chloride were added and 2-3 drops of gelatin salts reagent were added to the other. The occurrence of a blackish blue color in the first test tube and turbidity in the second test tube denotes the presence of tannins.

**Test for anthraquinone glycoside:**

0.2g of three extracts was boiled with 10ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinone was indicated if the alkaline

layer was found to have assumed pink or red color.

**Biological studies of plant:**

**Test microbial strains**

The standard microbial strains were provided from the culture collection used in the study was National Collection of Type Culture (NCTC), Colindale, England and American Type Culture Collection (ATCC), Rockville, Maryland, USA (Table 1).

**Table 1:** Tested microbial strains

| **Microorganism** | **Strain** |
|---|---|
| *Bacillus subtilis* | NCTC8236 |
| *Staphylococcus aureus* | ATCC25923 |
| *Escherichia coli* | ATCC25922 |
| *pseudomonas aeruginosa* | ATCC27853 |
| *Candida albicans* | ATCC7596 |

**Preparation of the test organisms:**

**Preparation of bacterial suspensions:**

One ml aliquots of a 24 hours' broth culture of the test organisms were aseptically distributed into nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about $10^8$-$10^9$ C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (24). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred using a micro pipette onto the surface of dried nutrient agar plates. The plates were left for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

**Preparation of fungal suspension:**

The fungal cultures were maintained on

Sabouraud dextrose agar, incubated at 25 ℃ for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

**Preparation of media**

**Nutrient agar**

The amount of nutrient agar was weighted and dissolved in distilled water, then heated under boiling temperature in water bath until it was completely dissolved, then sterilized in an autoclave (121°C\15mmHg).

***In vitro* testing of extracts for anti-bacterial activity**

The antimicrobial assay of plants extracts against different bacterial strains was conducted by disc diffusion method.

**Testing of Disc diffusion method**

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to $10^8$cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly in the surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (What man No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

**Antioxidant activity**

**DPPH radical scavenging assay**

The DPPH radical scavenging was determined according to the method of (25) with some modifications. In 96- wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl) -1- Picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as I (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517nm using multi plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

**Results**

Table (2) showed the results of extractive values of the plant for 70 % ethanol 1.4%

follow by petroleum ether 2.3% and finally ethyl acetate 3.1% respectively, as well as their physical appearance.

**Table (2)** Color, consistency and yield of *X. aethiopica* extracts

| **Solvents** | **Color of Extract** | **Consistency** | **Yield%** |
|---|---|---|---|
| Petroleum Ether | Black green | Oily | 2.3 |
| Ethyl acetate | Black Green | Sticky | 3.1 |
| 70 %Ethanol | Black | Sticky | 1.4 |

**Phytochemical screening of *X. aethiopica***

Phytochemical Screening of *X. aethiopica*to detects some secondary metabolites results are shown in Table (3).

**Table (3):** Chemical constituents of *Xylopia aethiopica* fruits

| **Extracts** | **Constituents** | **Test** | **Results** |
|---|---|---|---|
| troleum Ether | Alkaloids | Mayer's | - |
| | Tannins | FeCl3 | - |
| | Flavonoids | 1%Aluminum chloride+methanol | - |
| | Triterpenes | Aceticanhydride+H2SO | + |
| | Steroids | Aceticanhydride+H2SO | + |
| | Saponins | Distilled water | - |
| | Coumarine | **0.5**N KOH | - |
| | Alkaloids | Mayer's | - |

| | | | |
|---|---|---|---|
| Ethanol 70% | Tannins | FeCl3 | - |
| | Flavonoids | 1%Aluminum chloride+methanol | + |
| | Triterpenes | Aceticanhydride+H2SO | - |
| | Steroids | Aceticanhydride+H2SO | - |
| | Saponin | Distilled water | ++ |
| | Coumarine | **0.5**N KOH | + |
| | Alkaloids | Mayer's | - |
| | Tannins | FeCl3 | - |
| Ethyl acetate | Flavonoids | 1%Aluminum chloride+methanol | - |
| | Triterpenes | Aceticanhydride+H2SO | + |
| | Steroids | Aceticanhydride+H2SO | - |
| | Saponin | Distilled water | - |
| | Coumarins | **0.5**N KOH | - |

**Key:**+Trace ++Moderate +++High - Negative

According to phytochemical screening triterpenes, flavonoids, Steroids, coumarins and Saponin are present.

**Biological activities**

**Antimicrobial activity of *X. aethiopica***

The antibacterial activity of *X. aethiopica* extracts was investigated against standards microbial strains, two Gram positive (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram negative bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and one standard strain of fungi (*Candida albicans*) at concentrations of (100, 50, 25, 12.5) as shown in Table (4).

**Table (4):** Antimicrobial activity of *X. aethiopica*Fruits

| **Plant extract** | **Conc. Used** | **Standard tested organisms M.D.I.Z(mm)** | | | | |
|---|---|---|---|---|---|---|
| | | ***B.s*** | ***E.c*** | ***Ps.a*** | ***S.a*** | ***C.a*** |
| Ethanol | 100 | 19 | 21 | 19 | 18 | 16 |
| | 50 | 17 | 20 | 18 | 16 | 14 |
| | 25 | 16 | 16 | 17 | 16 | 15 |
| | 12.5 | 17 | 17 | 16 | 15 | 13 |
| Petroleum ether | 100 | 20 | 15 | 16 | 18 | 17 |
| | 50 | 17 | 12 | 14 | 18 | 17 |
| | 25 | 15 | 15 | 15 | 15 | 15 |
| | 12.5 | 19 | 15 | 12 | 14 | 15 |
| Ethyl acetate | 100 | 17 | 15 | 17 | 20 | 19 |
| | 50 | 16 | 20 | 15 | 15 | 18 |
| | 25 | 17 | 18 | 13 | 13 | 17 |
| | 12.5 | 15 | 16 | 14 | 17 | 17 |

*Standard tested organisms: B.s= *Bacillus subtilis*, E. coli =*Escherichia coli*, Ps. a= *Pseudomonas aeruginosa* , S. a= *Staphylococcus aureus* , C.a= *Candida albicans* .M.D.I.Z= Mean diameter of growth inhibition zone in (mm).

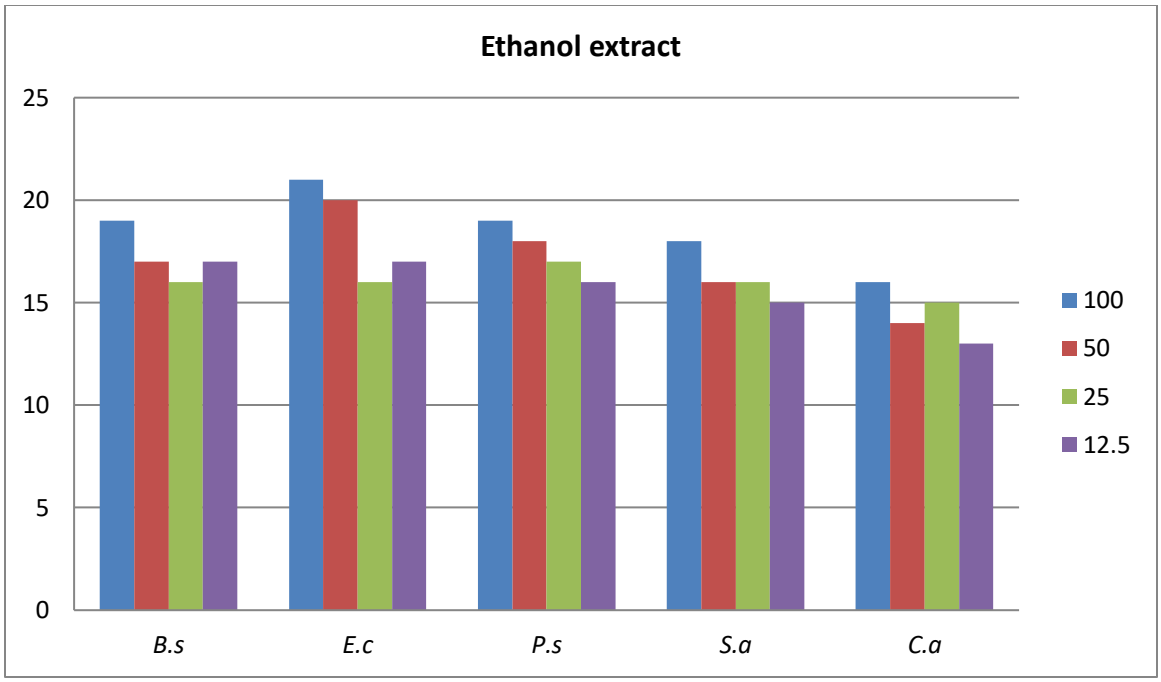



**Figure 1:** Antimicrobial activity of ethanol extract of *Xylopiaa ethiopica* fruit at Concentrations (100, 50, 25 and 12.5). Each bar represents zone of inhibition in diameters (mm).
B.s= *Bacillus subtilis*, E.c =*Escherichia coli*, Ps.a= *Pseudomonas aeruginosa* , S. a= *Staphylococcus aureus* , C.a= *Candida albicans*

The ethanol extract of the plant exhibited high activity against *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *pseudomonas aeruginosa* while it maintained moderate activity against *Candida albicans* (Fig 1).

The petroleum ether extract of the plant show high activity against *Bacillus subtilis* and, Staphylococcus aureus, while moderate activity against *E.coli*, *pseudomonas aeruginosa* and *Candida albicans* (Fig 2).

The ethyl acetate of extract of the plant reflected high activity against E. coli, *Staphylococcus aureus* and *Candida albicans*, and moderate activity against *Bacillus subtilis* and *pseudomonas aeruginosa* (Fig 3).

**Antioxidant activity of *X. aethiopica***

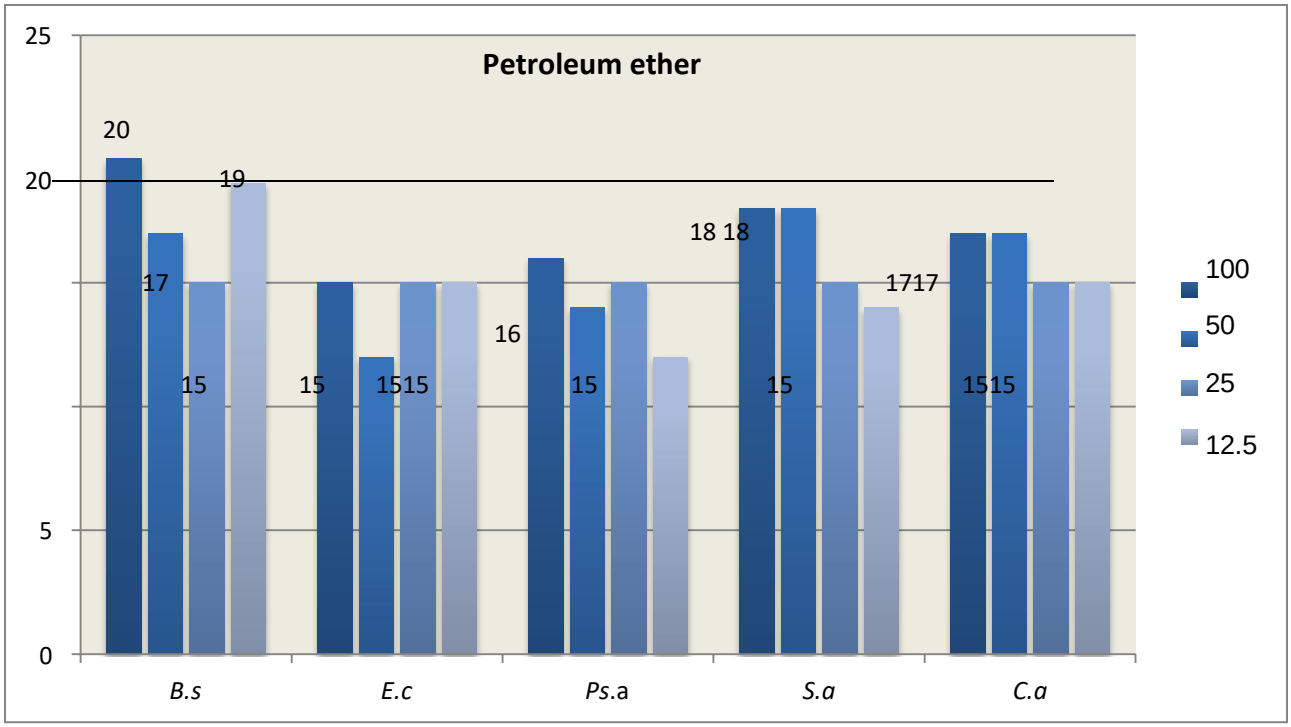
The antioxidant activity of different extracts of *Xylopia aethiopica* fruits have been evaluated using important parameters (% RSA±SD) free radical scavenging activity DPPH as shown in Table (5).

As indicated in Table (5), the extracts of *X. aethiopica* fruits showed moderate antioxidant activity for the ethanol and acetyl acetate extracts, while the petroleum

ether extract reflected low activity compared with the standard propylgallate. The histogram of the antioxidant activity for the different extracts is illustrated in Figure (4).

The ethanol and ethyl acetate extracts of the plant showed high antioxidant activity while the Petroleum ether showed low activity.

**Figure 2**: Antimicrobial activity of Petroleum ether extract of *Xylopia aethiopica* fruits at Concentrations (100, 50, 25 and 12.5). Each bar represents zone of inhibition in diameters (mm). B.s= *Bacillus subtilis*, E.c =*Escherichia coli*, Ps.a= *Pseudomonas aeruginosa* , S. a= *Staphylococcus aureus* , C.a= *Candida albicans*



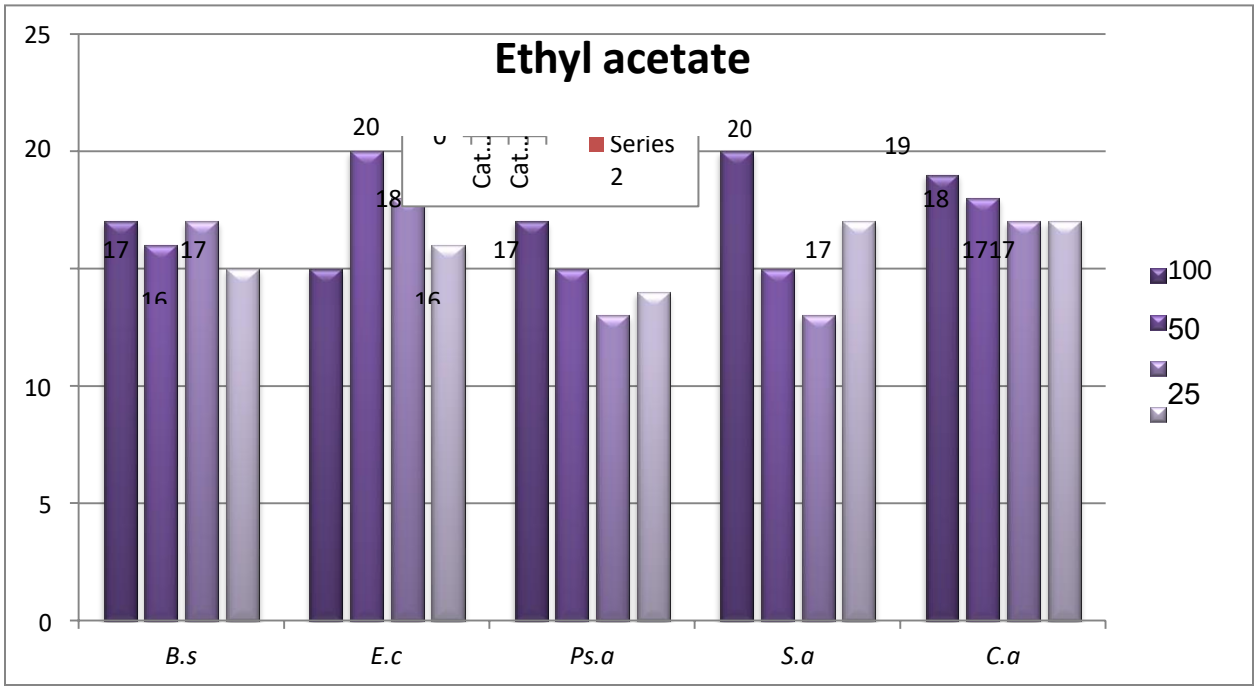



**Figure3:** Antimicrobial activity of Ethyl acetate extract of *Xylopia aethiopica* fruits at Concentrations (100, 50, 25 and 12.5). Each bar represents zone of inhibition in diameters (mm). B.s= *Bacillus subtilis*, E.c =*Escherichia coli*, Ps.a= *Pseudomonas aeruginosa* , S. a= *Staphylococcus aureus* , C.a= *Candida albicans*

| **Extracts** | **%RSA±SD(DPPH)** |
|---|---|
| Ethanol extract | 60±0.07 |
| Petroleum ether | 20±0.01 |
| Ethyl acetate | 56±0.05 |
| Propyl gallate(Standard) | 92±0.00 |

**Table (5)**: Antioxidant activity of *Xylopia aethiopica* extract using DPPH**:**

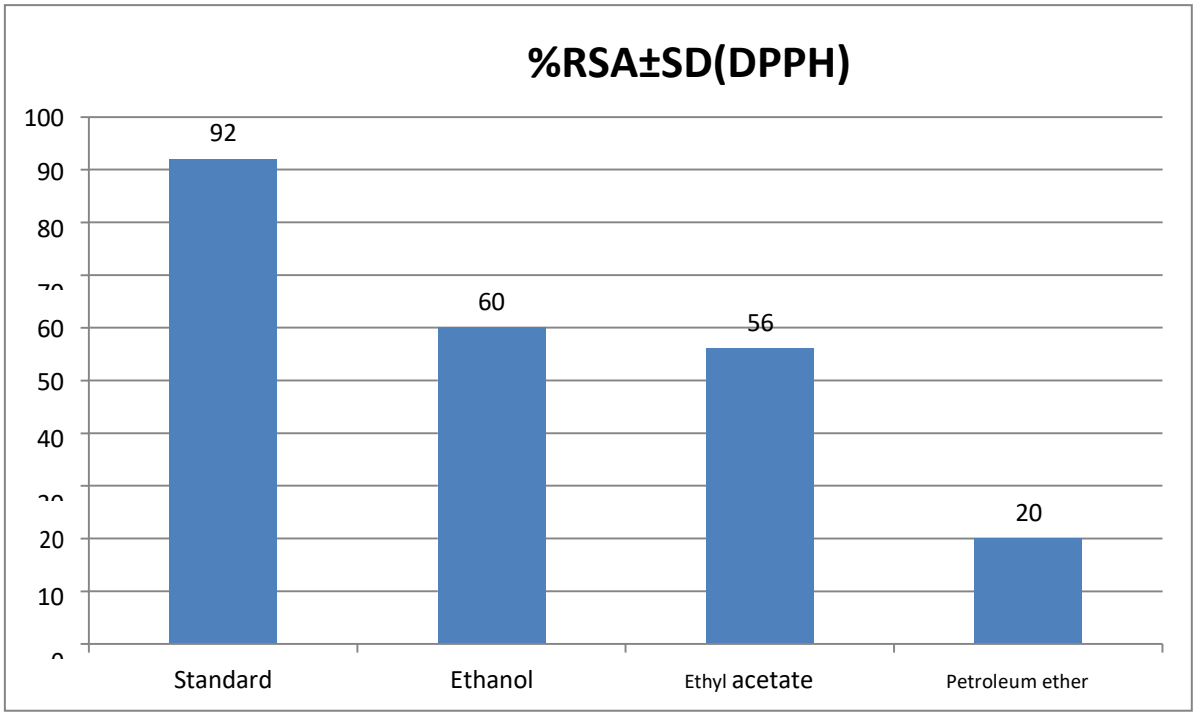



**Figure 4**: Antioxidant Activity of *Xylopia aethiopica* extracts by % RSA ± SD (DPPH).

*RSA: radical scavenging activity

## Discussion

The widespread use of common folk medicine is important and widely used in most countries around the world. In some Asian and African countries, about 80% of the population relies predominantly on plants and plant preparation for primary health care (26). Recently, the trend of using herbal medicines alongside pharmaceutical drugs has become popular in some Western countries. In addition, medicinal plants also offer significant economic benefits. The precise value of global markets for products derived from plants is difficult to estimate (27, 28). In this study on the fruit of *X. aethiopica*the phytochemical screening crush plant samples revealed the presence of alkaloids, saponins, coumarins, flavonoids, steroid and triterpines. Phytochemical screening results were similar to those obtained in a previous study for *X. aethiopica* fruits from Nigeria (29).

In antibacterial activity the extracts of X. *aethiopica* showed activity in all concentrations (100, 50, 25, 12.5) against all strains tested. On the other hand; all plant extracts exhibited antifungal activity against *Candida albicans.* The above results revealed that *X. aethiopica* has an excellent antimicrobial activity and can be used for disease therapy. The antimicrobial activity findings in this research are similar to those obtained in a previous study in which the ethanol extract was actively used against *P. aeruginosa*, *B. subtilis* and *S. aureus,* and differ from it in the in-activity of the extract towards E. *coli* in the previous study (29).

## Conclusion

This study results revealed that *X. aethiopica*is rich in secondary metabolites such as alkaloids, flavonoids, triterpenes, steroids, coumarins and saponins. Different extracts of *X. aethiopica* are recommended to be used against Gram-positive and Gram-negative bacteria, and as an antifungal agent. The *X. aethiopica*was found to be a good source for antioxidant.

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