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# Phytochemical Screening and *in vitro* Antibacterial Activity of *Cassia didymobotrya* Fres.

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## ABSTRACT

Man has used plants for treatment and prevention of diseases since prehistoric times. The cost of antibiotics coupled with bacterial resistance especially in developing countries like Kenya, has made it necessary to upscale research on cheaper antibiotics with novel mechanism of action. Studies have demonstrated a promising potential of antimicrobial agents from plants which are also likely to be cost effective. This study aimed at investigating the phytochemical constituents and the antibacterial activity of methanolic and aqueous extracts of Cassia didymobotrya Fres. leaves and stem bark. Preliminary phytochemical screening was done using established protocols while antibacterial activity was by the Agar Well Diffusion Method and assessment of zones of inhibition. E. coli and P. aeruginosa were the most susceptible organisms while S. aureus was the least susceptible. Leaf extracts generally showed better activity than the stem bark extracts.

***Keywords:*** phytochemical screening, zones of inhibition, Cassia didymobotrya Fres.

## INTRODUCTION

Up to 90% of the populations in certain African countries still rely exclusively on plants as source of medicine [1] while in some parts of the world, many people use traditional and complementary medicine, herbal medicines, traditional treatments and traditional practitioners are the main source of health care, and some instances, the only source of care[2] Many developing countries, including Kenya, do not have sufficient technological advancement and investment in pharmaceutical manufacturing and rely on imported conventional drugs. Besides, they also have few healthcare providers with Kenya reporting 25 medical officers per 100,000 people in the population [3] The cost and side effects of conventional drugs coupled with insufficient and inaccessible health care facilities and health personnel has led to overreliance on traditional medicine in these countries since it is affordable, reliable, available and culturally acceptable to rural populations [4]

Microbial infections are currently a major public health problem all over the world. Factors like medical complications arising from HIV and other virus pandemics, overcrowding, poor hygiene and resistance to conventional drugs make the global situation dire. [5] Ethno pharmacological surveys and studies of antimicrobial active principles from medicinal plants are important since more than 25% of current drugs having been derived from plants. [6] The aim of this study was to identify the phytochemical constituents and assess the in vitro antibacterial activity of Cassia didymobotrya Fres. leaf and stem bark

## LITERATURE REVIEW

The use of plants and other natural products for management of diseases is ancient. Medicinal plants comprise much of traditional medicine practice globally. In many instances, traditional medicine is used side by side with conventional medicine

depending on the cost and availability. [7] Kenya's healthcare system is already overwhelmed because of increase in demand in regions where universal health coverage has been implemented. This in turn has led to high costs of operations, overworked staff and low staff morale making the health facilities are unable to cope with the situation. There is also a global increase in research regarding traditional remedies. All the above including side effects associated with conventional drugs have served to give a lifeline to traditional medicine in Kenya. [4] It is estimated that over 25% of current drugs have been derived from plants. [6] Among the plants with medicinal value include genus Cassia.

**Cassia didymobotrya**

Cassia is a large genus in the family Fabaceae. Cassia species are used for their cathartic, purgative and antibiotic properties. They are also sources of polysaccharides, anthraquinones, mucilage and flavonoids while others yield timber, tannins and dyes, fodder, vegetables, edible fruits, and seeds. [8]

Cassia didymobotrya Fres. is known by common names like African senna, popcorn senna, wild senna, and peanut butter cassia. In Kenya, different communities have different names for Cassia didymobotrya Fres. such as ithaa (Kamba), lubino (Luhya), kilao (Meru), mshua (Taita), mwino; (Kik), omovenyu (Gusii), owinu (Luo), ol-senetoi (Mas), senetwet (Kipsigis) [9]

It is used in management of malaria, abscesses, ringworms, gonorrhea, stomachaches, headaches, colds and fever / flu. [9,10] In addition, it is used to treat skin conditions in human and livestock. Its other uses include as firewood mulching, as shade, dying, in ornamental and home gardens. [11] Scientific validation is important to verify claims of medicinal efficacy of plants even when they have been used for long periods. [12] Such tests should be carried out under conditions mimicking the indigenous methods of use. [13] Phytochemical screening identifies the secondary metabolites which are responsible for the plants' medicinal activity. These include enzymes like papain, alkaloids like morphine, phenols like capsaicin, flavonoids like hesperidin, among many others. [14] Assessing plants for phytochemicals should be a continuous process and is important since it can lead to identification of new compounds which had not been previously identified or attributed to that particular plant. [1]

Antimicrobial assays of plant extracts are necessary because greater than 70% of bacteria causing infections are resistant to at least one of the drugs commonly used to treat them. [15] The compounds in plants can act as lead compounds for drug design and development or as markers for standardization of antimicrobial herbal remedies. [1] Phytochemical screening and antimicrobial assay were thus used to evaluate the antimicrobial activity of Cassia didymobotrya Fres.

## MATERIALS & METHODS

The research was carried out in Kenyatta University main campus. The investigated plant species was chosen based on the ethnobotanical information regarding its use as an antibacterial plant species. Being a shrub, the plant does well in the open savannah climate within the campus and its environs and thus this was considered a viable study area due to the high density of the study plant species in the region. The research was a laboratory-based experiment that aimed at identifying the phytochemical constituents and antibacterial activity of Cassia didymobotrya Fres. leaf and stem bark extracts.

**Collection and Identification of plant material**

The plant material collection was done in ''kilometer one'' area which is about a kilometer from the main campus. This was done during the optimal season of the months of September and November

when its secondary metabolites are abundant. [16] Identification of the plant was done in the field and laboratory by a plant taxonomist from the department of pharmacognosy and pharmaceutical chemistry in Kenyatta University, after which a voucher specimen was deposited in the university's herbarium.

**Cassia didymobotrya Fres. shrub**

**Preparation of material**

The leaves and the stem bark were transported to the campus in clean polythene bags from where they were washed with distilled water to remove any foreign matter then dried under shade in the Kenyatta University's school of pharmacy laboratories. On drying the materials were ground to a fine powder using a laboratory mill in a fume chamber to protect from fumes and dust during the grinding and the powder obtained was packed in clean airtight polythene papers

**Extraction**

The procedure for extraction was adopted from Musau et al., (2016) [16] with slight modifications. For methanol extraction one hundred grams (100 g ) of the powdered leaves and the stem bark was separately placed in 1000 ml conical flasks to which methanol and water was added in the ratio of 7:3 respectively until the materials were completely submerged in the solvent. The flasks were covered with an aluminum foil and kept in a shaker for 72 hours for complete extraction of active materials. The extracts were then filtered by use of a muslin cloth followed by Whatman No. 1 filter paper after which the solvent was removed using a Rota evaporator and the liquid extract stored in a refrigerator. For aqueous extraction, 800 ml of water was added to 100 mg of plant materials each in a separate conical flask and macerated for 48 hours after which they were filtered using a Muslin cloth and Whatman No 1 filter paper and then freeze dried to obtain dry powders. The percentage yield for each form of extraction was calculated by:

$$\frac{\text{Weight of concentrate}}{\text{Weight of dry powder added}} \times 100 = \% \text{ Yield}$$

**Phytochemical screening**

Phytochemical screening was done according to established procedures adopted by Musau, 2011. [17] The extracts and the dry powders were screened for the presence or absence of saponins, tannins, flavonoids, terpenes, alkaloids, phenols, anthracenes and anthraquinones.

**Test for alkaloids**

100mg of the extract was dissolved in 0.2 ml 1% sulphuric acid and to about 0.1 ml proportion of the solutions a single drop of Mayer's reagent added. Appearance of cream and orange precipitates indicated the presence of alkaloids.

**Test for flavonoids**

Shinoda Test: to the extract, about 3 gms of magnesium powder was added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple color indicated the presence of flavonoids.

**Test for saponins**

About 100 mg of the extract was shaken with water in a test tube. Frothing that persisted for 15_minutes indicated the presence of saponins.

**Test for tannins**

Ferric chloride test: a small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride were added to the filtrate, formation of a blue black, or green precipitate indicated evidence of tannins.

**Test for terpenes**

Liebermann-burchard Test: 1ml of anhydrous acetic acid was added to 1ml chloroform and cooled to 8 degrees centigrade. A drop of concentrated sulphuric acid was added to the cooled mixture followed by the extract. The solution was observed for blue, green, or orange color that changes with time.

**Test for anthraquinones**

About 100 mg of plant extract was shaken with 10 ml of benzene, filtered, and then 5 ml of 10% ammonia was added to the filtrate. Pink color indicated presence of anthraquinones.

**Modified Borntrager's test (test for anthraquinones)**

A few drops of 5% iron (iii) chloride were added during extraction with dilute ammonia. A rose-pink color in the ammoniacal layer indicated presence of anthracene a glycone in reduced state.

**Test for phenolic compounds**

About 25 mg of the extract was dissolved in a mixture of water and ethanol, and a few drops of dilute (5%) ferric chloride added. Formation of a faint green color indicated presence of phenols.

**Preparation of solutions for antibacterial testing**

One gram of the plant extract obtained after concentration of the filtrate was placed in a test-tube. One milliliter of DMSO was added into the test tube and heated gently over a water bath to facilitate the dissolution of the plant extract in DMSO. Serial dilution was then done by pipetting 0.5 ml into a second test tube and adding 0.5mls of DMSO. This was repeated until 3 different concentrations were obtained. The positive controls were the antibiotics Ampicillin sodium for gram positive bacteria and Gentamicin sulphate for gram negative bacteria. 2% DMSO was used as negative control [18, 17]

**Antibacterial activity testing**

The methanolic and aqueous extracts were screened for their antibacterial activity using the agar well diffusion method. The gram-positive bacterial strain was Staphylococcus aureus (ATCC29213) and the gram-negative bacterial strains were Escherichia coli (ATCC25922) and Pseudomona aeruginosa (ATCC27853). They were obtained from stock cultures at the department of medical microbiology and parasitology of Kenyatta University.

**Culture medium preparation and inoculation**

Mueller-Hinton Agar was prepared according to the manufacturer's instructions, autoclaved and 20 ml dispensed per plate in 12 x 12 cm Petri dishes. The plates were then incubated overnight to ensure sterility before use. Suspension of micro-organisms were made in peptone water and adjusted to 0.5 Macfarland standards ($10^8$Cfu/ml) (NCCLS, 2000). Each labeled medium plate was uniformly inoculated with a test organism using an inoculation loop.

A sterile cork borer was used to bore wells of 6mm in diameter and equidistance from each other in the inoculated plates. 50 µl of the extract's serial dilutions (methanolic and aqueous extracts) were put in this wells. The plates were then refrigerated for 1 hour to allow the extracts to diffuse into the agar after which they were incubated at $37^0$C for 24 hours. Gentamicin and Ampicillin were used as standard control drugs for the gram negative and gram-positive bacterial strains respectively. Antibacterial activity was determined by measuring the diameter of zones of inhibition (clear zones) produced after incubation using a ruler (mm).

**Statistical Analysis:** Data analysis was done using Microsoft excel ®.

## RESULTS

**Percentage yield**

**C**assia didymobotrya leaf extracts

| Methanolic extract | Aqueous extract |
|---|---|
| Weight of concentrate= 10.81g | Weight of concentrate=2.51g |
| Weight of dry powder= 100g | Weight of dry powder=100g |
| Percentage yield = 10.81% | Percentage yield=2.51% |

**C**assia didymobotrya stem bark extracts

| Methanolic extract | Aqueous extract |
|---|---|
| Weight of concentrate= 9.81 | Weight of concentrate=1.11g |
| Weight of dry powder= 100g | Weight of dry powder=100g |
| Percentage yield= 9.81% | Percentage yield=1.11% |

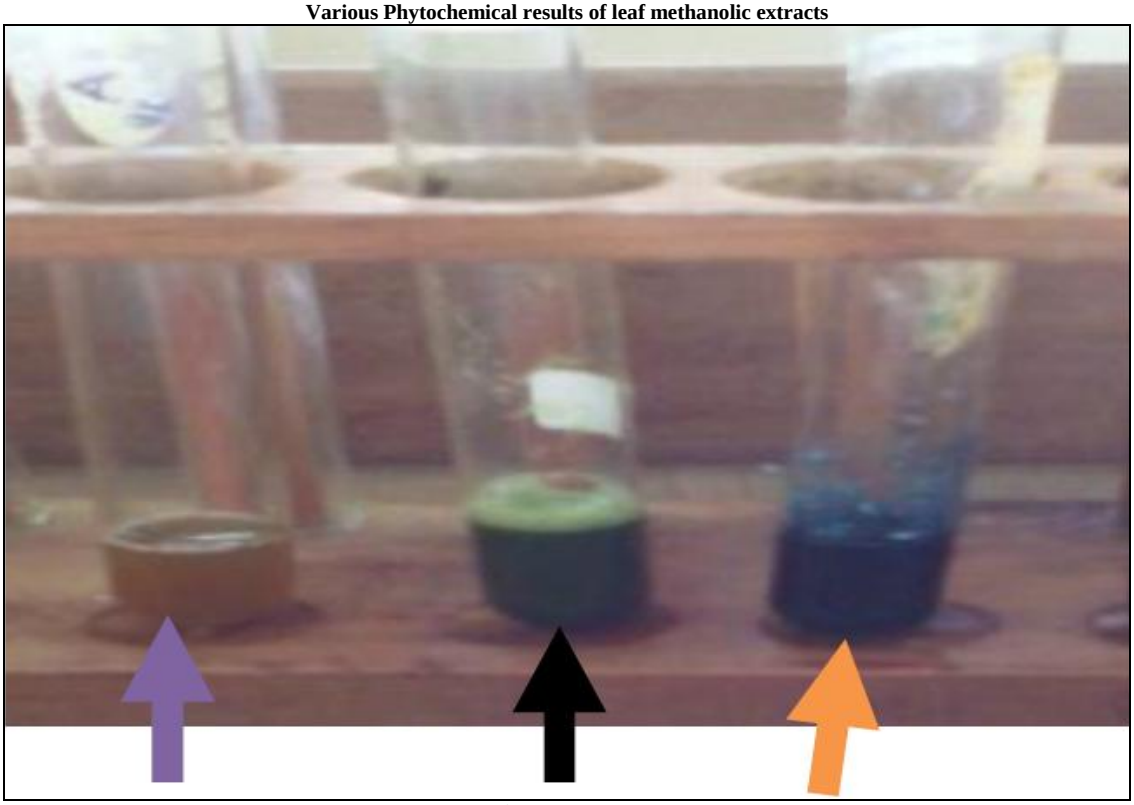
**Phytochemical screening tests**

Phytochemical Results for Cassia didmobotrya Extracts

| | Stem bark | | Leaf | |
|---|---|---|---|---|
| **Compound** | **Aqueous** | **Methanol** | **Aqueous** | **Methanol** |
| Alkaloids | + | + | _ | _ |
| Saponins | + | + | + | + |
| Flavanoids | + | + | + | + |
| Tannins | + | + | + | + |
| Terpenes | + | + | + | + |
| Phenols | + | + | + | + |
| Anthracenes | + | + | + | + |
| Anthraquinones | _ | _ | _ | _ |

+: presence of secondary metabolite
-: absence of the secondary metabolite

**Various Phytochemical results of leaf methanolic extracts**



Key: Orange arrow: presence of tannins indicated by the blue black coloration
Black arrow: presence of saponins indicated by persistence foaming that persisted for 15 minutes
Purple arrow: presence of flavanoids indicated by orange coloration

## Antibacterial activity testing

### Zones of inhibition

Antibacterial activities were assessed in terms of zone of inhibition after overnight incubation of the culture. The diameter of the inhibition zone was measured from the edge of the disc to the edge of the zone. The end point of inhibition is where growth starts.

Zones of inhibition for C. didymobotrya leaf extracts and the positive controls

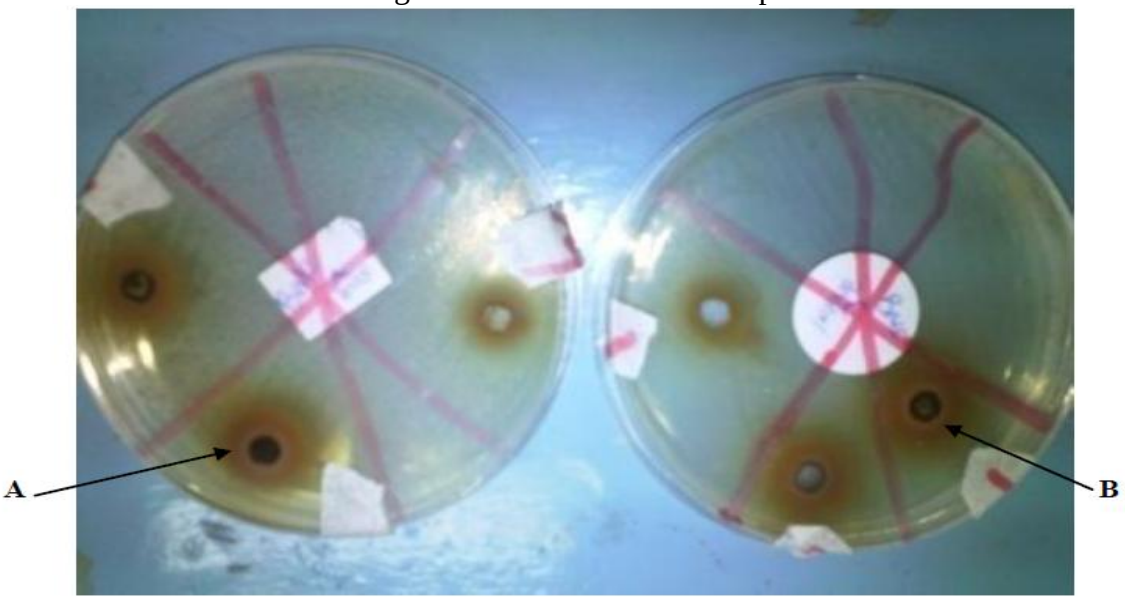
| | | Zones of Inhibition (mm) | | |
|---|---|---|---|---|
| | | **S.aureus (ATCC29213)** | **P.aeruginosa (ATCC27853)** | **E.coli (ATCC25922)** |
| Methanolic Extract Concentrations | 1g/ml | 24.0 | 25.5 | 29.5 |
| | 0.5g/ml | 16.5 | 17.0 | 18.0 |
| | 0.25g/ml | 7.0 | 7.0 | 8.5 |
| Aqueous Extract Concentrations | 1g/ml | 29.5 | 16.5 | 21.5 |
| | 0.5g/ml | 21.5 | 12.0 | 15.5 |
| | 0.25g/ml | 7.5 | 6.5 | 2.0 |
| Ampicillin | 10 µg | 30 mm | 10 mm | resistant |
| Gentamycin | 10 µg | 12 mm | 25 mm | 22 mm |

**Zones of inhibition for C.didmobotrya stem bark extracts and the controls**

| | | Zones of inhibition(mm) | | |
|---|---|---|---|---|
| | | **S.aureus (ATCC29213)** | **P.aeruginosa (ATCC27853)** | **E.coli (ATCC25922)** |
| Methanolic Extract Concentrations | 1g/ml | 17.5 mm | 20.5 mm | 16.5 mm |
| | 0.5g/ml | 13.5 mm | 16.0 mm | 13 mm |
| | 0.25g/ml | 9.0 mm | 9.5 mm | 9.5 mm |
| Aqueous Extract Concentrations | 1g/ml | 16.5 mm | 19.0 mm | 14.5 mm |
| | 0.5g/ml | 12.5 mm | 13.5 mm | 12 mm |
| | 0.25g/ml | 5.5 mm | 7.0 mm | 6.0 mm |
| Ampicillin | 10 µg | 30 mm | 10 mm | resistant |
| Gentamycin | 10 µg | 12 mm | 25 mm | 22 mm |

A Petridish showing zones of inhibition for the standard disc, which contained among others Ampicillin and Gentamicin 10 µg respectively

Arrows showing zones of inhibition for the plant extracts




## DISCUSSION

Bioactivity of plants is dependent on their secondary metabolites. The effect achieved from crude extracts of plants is due to the overall content of the substances which may act synergistically hence their

overall bioactivity is thus greater than for individual compound. [19.20] The secondary metabolites present were alkaloids, saponins, flavonoids, tannins, terpenes, phenols and anthracenes. Tannins have astringent property and antimicrobial activity. [21] Alkaloids are haemolytically active, toxic to some micro-organisms and may interfere with cell division. [22,23] Saponins have antibacterial activity by increasing permeability of cell membranes. [24]

In this study, the antibacterial activity of the extracts increased linearly with increase in concentration of extracts (g/mls). E.coli showed highest susceptibility for the methanolic leaf extract followed by P.aeruginosa. these two organisms are the major causes of opportunistic infections with P.aeruginosa showing resistant to many antibiotics. as such, this finding is vital. In the aqueous extract, S.aureus showed the highest susceptibility which was greater than for the methanolic exract while P.aeruginosa showed the least susceptibility. The increased activity of the methanolic extract is probably because of its high capacity to extract both polar and non polar compounds resulting in more active compounds in these extracts. The action of the aqueous extract can be ascribed to the anionic components e.g. nitrates and chlorides together with other water soluble compounds that are naturally occurring in plants. [20]

The zones of inhibition of both the methanolic and aqueous stem bark extracts did not differ significantly from each other. P.aeruginosa and E.coli showed the highest and lowest susceptibility respectively. This supports use of the plant by herbalists, as many use water as the solvent for extraction. Leaf extract showed more activity than the stem bark extract despite the absence of alkaloids. This could be due to presence of tannins since tannins have been shown to have antimicrobial activity through molecular inhibitions that mostly occur in the cell membrane of microorganisms, such as the inhibition of the formation of complexes that maintain its integrity, generating malformations and increasing their permeability. [21] Use of leaves allow for sustainable harvesting due to their quick regeneration. Stem bark harvesting is destructive to the plants and can not be sustained in long term. As compared with standard drugs, the results revealed that the gram negative bacteria were more susceptible to the plant extract than the gram positive bacteria.

## CONCLUSION

The use of medicinal plants in treatment and prevention of diseases caused by microbes needs to be backed by scientific validation and regulation in order to avoid toxicity and further development of resistance. The presence, type and quantity of secondary metabolites in a plant determine its actions which could be beneficial or harmful. The methanolic and aqueous extracts of Cassia didymobotrya possessed antibacterial activity with the leaf extracts showing greater activity than the stem bark extracts. E.coli and P. aeruginosa were more susceptible to the plant extracts than S.aureus. This study lends some credence to the claimed uses of the plant in folk medicine to treat various bacterial infections. However, it's use in general bacterial infection need to be guided by use of more scientific evaluations and studies on the plant.

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