The evaluation of the antimicrobial property of *Cassia alata* leaves in Benin city, Nigeria

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Abstract: *Cassia alata,* a tropical plant is claimed to have a wide range of therapeutic values. The plant is commonly used traditionally for the treatment of a wide variety of diseases. This study focused on the evaluation of the antimicrobial properties of the ethanol and aqueous extracts of *Cassia alata* leaves. The leaves were subjected to ethanol and aqueous extraction after which phytochemical screening was done to determine the phyto-constituents (bioactive compounds) in the leave extracts. The leaf extracts were investigated for it's antimicrobial property. Agar diffusion method was used for the antimicrobial evaluation on muella-hinton agar for the bacteria and potato dextrose agar for the fungi. The preliminary phytochemical screening revealed the presence of cardiac glycoside, reduced sugar in equal concentration in both extracts while flavonoids, terpenoids, saponins and phenolics were in slightly higher concentrations in the ethanol extract than in the aqueous extract. Different concentrations of both extracts were tested on both bacteria and fungi. The antimicrobial effects produced by the extracts were dose dependent at the tested doses; 1000mg/ml, 500mg/ml and 250mg/ml. Only the aqueous extract showed activity against *Escherichia coli* at 1000mg/ml and 500mg/ml concentrations whereas the ethanol extract showed higher activity than the aqueous extract at 1000mg/ml and 500mg/ml concentrations against *Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus* and at 1000mg/ml, 500mg/ml and 250mg/ml against *Klebsiella pneumoniae*. Only the ethanol extract showed activity against Trichonphyton species at 1000mg/ml and 500mg/ml and *Alternaria alternata* at 1000mg/ml, 500mg/ml, 250mg/ml and 125mg/ml concentrations while Aspergillus species was resistant to all the concentrations of both the ethanol and aqueous extracts. This study showed that the aqueous and ethanol extracts of *Cassia alata* leaves contain some antimicrobial properties, and thus support the traditional use of this plant in the treatment of infections caused by micro-organisms.
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Key words: Cassia alata; antimicrobial activity; aqueous and ethanol extracts; phyto-constituents

Introduction

The use of plants for the treatment of certain illnesses dates back to many centuries, by our ancestors who not only used plants as food and shelter but also for the treatment of certain illnesses (Akinyemi et al., 2000). Plants serve as food for man and animals, since man and animals cannot manufacture their own food and so rely solely on plants to provide them with nutrients such as vitamins, proteins, carbohydrates, lipids and essential minerals that are required for growth, development and proper body functions, (Selvi et al., 2012).

Apart from the nutrients provided by plants, man discovered that by consuming some plants their health conditions improved. This gave rise to the medicinal use of plants and it was discovered that some of these plants contained certain chemical substances that have therapeutic values (Masuda *et al.,* 2003). Plant parts used for medicinal purposes vary among plant species and also dependent on the location of the medically active components. Some plants have their active component in their roots, others in their leaves while some others bear theirs in their seed, fruits, and flowers or stem bark, (Selvi et al., 2012).

The increasing use of plant for the production of natural products for maintaining human health which has been observed over the last decades, has led to the increased studies by scientists to discover more medicinal plants, and according to the World Health Organization (WHO), medicinal plants would be the best source of a variety of drugs as 80% of individuals in developing countries use traditional medicine, which has compounds derived from medicinal plants, (W.H.O., 2006).

One of such plants is *Cassia alata* an erect tropical perennial herb that grows up to 6ft tall and it belongs to the family; fabaceae. It is a plant originally grown in South America but due to its medicinal value, is now found in other countries, such as India where it is called Dadmari and in Nigeria it is called Rai-dore in Hausa, Asuwon oyinbo in Yoruba and Omirima in Igbo, (Arbonnier, 2004). *Cassia alata* is used in traditional medicine for the treatment of a variety of infections and diseases including ringworm,

eczema, wounds, constipation, burns and food poisoning, hemorrhoids, inguinal hernia, intestinal parasitosis, syphilis and diabetes; and the leaves have also been reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and also used as a laxative (Zhongguo, 2009 and Adebayo *et al.,* 2001).

It was reported that ethanol leaf extracts of the *Cassia alata* plant showed high activity against dermophytes, like *Trichophyton mentagrophytes* which attacks the skin and scalp of man and *Trichophyton rubrum* and *Microsporium gypseum* and *Microsporium canis*, (Owoyale *et al.,* 2005) and the aqueous leaf extracts showed higher activity on *Escherichia coli* than the ethanol extract (Timothy *et al.,* 2012). The methanol extracts of Cassia alata leaves, flowers, stem and root showed a range of antimicrobial activity against *Bacillus cereus, Micrococcus luteus, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Neisseria gonorrhea, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens* and *Trichomonas vaginalis* (Somchit et al.,2003).

Several traditional uses of *Cassia alata* have been reported in several places. In Indo China and Philippines, the leaves are considered most effective against herpes and is also used as a mild laxative. In Guinea, the pounded fresh leaves are used for all kinds of skin infections. In Ghana, the leaves are used in the treatment of lesions and ringworm on the forehead or on the skin. This is one of the most effective treatments amongst traditional medicines. The leaves are also boiled and drunk by women to hasten delivery during birth, (Alalor, *et al.,* 2012).

Despite the numerous reports in the literature of the antimicrobial activity of *Cassia alata*, not much work has been reported from Nigeria. It is the paucity of information as regards this plant that necessitates this study, the need of which cannot be over emphasized. This study, therefore is aimed at evaluating the antimicrobial activity of the aqueous and ethanol extracts of *Cassia alata* leaves on clinically important micro-organisms such as, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Trichophyton species, Aspergillus species* and *Alternaria species* and also to encourage the medicinal use of plants in the treatment of infections caused by micro-organisms.

Materials And Methods

This study was conducted in the laboratory of Benson Idahosa University, in Benin city, Edo State of Nigeria. This is a private tertiary institution with student population of over four thousand. It has one of the best University laboratories equipped with modern facilities in Nigeria. The laboratory is designed and equipped for both graduate and undergraduate studies. The test organisms used for this research work were five clinical bacterial and three fungal isolates which were obtained from University of Benin Teaching Hospital (UBTH) a tertiary health institution in Benin city Nigeria. The clinical isolates are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsellia pneumoniea, Proteus vulgaris, Aspergillus species,, Alternaria species* and *Trichonphyton species*. These isolates were obtained in pure cultures from the Microbiology department of the University of Benin Teaching Hospital, Benin city and maintained in nutrient agar slants at 4°c.

All the media used in this study were prepared according to manufacturer's instructions, and they include:

- **Nutrient Agar:** A general medium used for isolation and enumeration of bacteria.
- **Potato Dextrose Agar (PDA):** A general media used for isolation of fungi.
- **Peptone water:** This medium was used to enrich and maintain the bacterial isolates for some biochemical test.
- **Nutrient broth:** This medium was used to enrich the bacterial isolates before they were inoculated onto Muella-Hinton agar plates for the antibacterial susceptibility.

The work bench was sterilized by cleaning with swab (cotton wool soaked in 75% alcohol). The liquid and solid media were sterilized by autoclaving at 121°c for 15mintues and were allowed to cool to 45°c before they were used. Glass wares such as test tubes, conical flasks, beakers and McCartney bottles were sterilized in the oven at 160°c for 1hour and were allowed to cool before they were used. The inoculating loop was sterilized by holding the chrome wire in the flame until it glowed red and was allowed to cool before use.

The fresh *Cassia alata* leaves used for this research were collected from mature *Cassia alata* plant at the botanical garden, faculty of Pharmacy, University of Benin, Ugbowo campus, Benin City, Edo state, Nigeria, and were taken to the microbiology laboratory of Benson Idahosa University located at Ugbor road, Benin City, Edo state, Nigeria for processing.

Fig 1: Showing *Cassia alata* plant from collection site (Faculty of Pharmacy, University of Benin, Ugbowo campus, Benin City, Edo State).

Aqueous And Ethanol Extraction Process

The leaves of *Cassia alata* were washed with clean water to remove unwanted particles, sun dried, ground to powder using mortar and pestle and sieved to obtain fine powder, using the method described by Getahun et al., (2014).

200 grams each of the powdered leaves was weighed and put into two containers labeled A and B. 2000mls each of distilled water (in container labeled A) and ethanol (in container labeled B) were added to the powdered leaves, stirred and kept in the refrigerator for 72hours, after which they were filtered and the filtrates were evaporated in an oven at 40°c to form semi-paste (crude extracts). The crude extracts were then preserved at 4°c in airtight sterile bottles until subsequent use.

Phyto-chemical screening:

Preliminary phyto-chemical analysis of the powdered plant material was carried out to screen for the chemical constituents to detect the major secondary metabolites present in the plant by using the standard method described by Sofowora (1993).

Preparation of the various concentrations of the aqueous and ethanol extracts

Preparation of the various concentrations of the extracts was done using serial dilution. 2g of the extract was weighed and dissolved into 2mls of distilled water in a sterile bottle, mixed using a vortex mixer to form the original concentration (1000mg) and was serially diluted (using 1ml as the dilution factor) into 6 different sterile bottles with each containing 1ml of distilled water using a sterile syringe and a vortex mixer to obtain the following concentration; 500mg, 250mg, 125mg, 62.5mg, 31.25mg, 15.63mg.

Preparation Of Turbidity Standard

To standardize the inoculums (bacterial isolates) density for the antibacterial assay, a BaSO4 turbidity standard, equivalent to a 0.5 McFarland or its optical equivalent was prepared. A 0.5ml aliquot of 0..048ml/L BaCL2 (1.175% w/v BaCL2, 2H2O) was added to 99.5ml of 0.18ml/L H2SO4 (1% w/v) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1cm light path and matched cuvette to determine the absorbance. The absorbance at 625mm should be 0.008 to 0.10 for the 0.5 McFarland standard and the turbidity correspond to 108cfu (Britz and Robinson, 2008).

Standardization of bacteria isolates

The bacterial isolates were inoculated into test tubes containing sterile nutrient broth. The test tubes were incubated at 37c for 24hours and were then matched with the prepared 0.5 McFarland standard by adjusting the turbidity of the bacterial suspension with dilute sterile nutrient broth.

Antibacterial assay

Antibacterial activity of the extracts and fractions were tested using the agar diffusion method. The varying concentrations of the extracts were tested using Muella-Hinton agar. The medium was autoclaved at 121°c for 15mins and allowed to cool (warm temperature), then swab stick was dipped into nutrient broth in test tubes containing the standardized test organism and used to spread on the solidified nutrient agar plates. A sterile cork borer was then used to pore 2 holes in each agar plate on which a sterile syringe was used to place 0.2mls of the various concentrations of the extracts (1000mg, 500mg, 250mg, 125mg, 62.5mg, 31.25mg, 15.623mg) and one hour was allowed for diffusion before the plates were incubated at 37°c for 24 hours and the zones of inhibition measured using a meter rule.

Antifungal assay

Antifungal activity of the extracts and fractions were tested using the agar diffusion method. Varying concentrations of the extracts were tested using Potato Dextrose agar. The medium was autoclaved at 121°cfor 15mins and allowed to cool (warm temperature) and 0.5mls of streptomycin solution was added to medium to prevent the growth of bacteria, then a sterilized inoculating loop was used to pick from the fungal culture and streaked on the solidified Potato Dextrose agar plates. A sterile cork borer was then used to pore 3 holes in each agar plate on which a sterile syringe was used to place 0.2mls of the various concentrations of the extracts (1000mg, 500mg, 250mg, 125mg, 62.5mg, 31.25mg, 15.623mg) and one hour was allowed for diffusion before the plates were incubated at 37^{0c} for 48hours and the zones of inhibition measured using a meter rule.

Statistical analysis:

The data were calculated using Microsoft-excel 2003, and expressed as means $\pm$ SD for each dose level. Data were analyzed using SPSS version 13 for windows software. Statistical analysis was done by one-way analysis variance (ANOVA) test coupled to Least Significant Difference (LSD) to compare results between doses. The result was considered statistically significant at 95% confidence level and p-value<0.05.

Results: The preliminary phyto-chemical screening revealed the presence of cardiac glycoside, reduced sugar in equal concentrations in both extracts while flavonoids, terpenoids, tannins, alkaloids, saponins and phenolics were slightly higher in concentration in the ethanol extract than in the aqueous extract.

Antibacterial Activity Of The Aqueous Extracts.

The aqueous extract of *Cassia alata* leaves showed zones of growth inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris*, *Escherichia coli* at 1000mg/ml and 500mg/ml, against *Klebsiella pneumoniea* at 1000mg/ml, 500mg/ml and 250mg/ml concentrations with 1000mg/ml concentration showing higher zone of growth inhibitions, whereas, at 125mg/ml, 62.5mg/ml, 31.3mg/ml and 15.6mg/ml concentration, there were no zones of growth of inhibition against all the organisms. The highest and lowest zones of growth inhibition were seen at the 1000mg/ml and 500mg/ml against *Klebsiella pneumionae* and *Staphlococcus aureus* respectively (Table 1).

Antibacterial Activity Of The Ethanol Extract.

The Ethanol extract of *Cassia alata* leaves showed zones of growth inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniea* at 1000mg/ml and 500mg/ml and against *Proteus vulgaris* at 1000mg/ml, 500mg/ml and 250mg/ml concentrations with 1000mg/ml concentration showing higher zone of growth inhibitions, whereas, at 125mg/ml, 62.5mg/ml, 31.3mg/ml and 15.6mg/ml concentration, there were no zones of growth of inhibition against all the organisms. There were no zones of growth inhibition against *Escherichia coli* at any of the concentrations.

The highest and lowest zones of growth inhibitions were seen at 1000mg/ml and 250mg/ml against *Klebsiella pneumionae* and *Pseudomonas aeruginosa* respectively (Table 2).

Antifungal Activity Of The Aqueous Extracts Of *Cassia Alata* Leaf

All the concentrations of the aqueous extract of *Cassia alata* leaves showed no zones of growth inhibition against all the fungal isolates, (Table 3).

Antifungal Activity Of The Ethanol Extract.

The ethanol extract of *Cassia alata* leaves showed zones of growth inhibition against *Trichonphyton sp.* at 1000mg/ml and 500mg/ml and against *Alternaria sp.* at 1000mg/ml, 500mg/ml, 250mg/ml and 125mg/ml concentrations, whereas, at 62.5mg/ml, 31.3mg/ml and 15.6mg/ml concentration, there were no zones of growth of inhibition against all the organisms. There were no zones of growth inhibition against *Aspergillus sp.* at any of the concentration. The highest and lowest zones of growth inhibition were seen at 1000mg/ml and 500mg/ml for Trichophyton species and 1000mg/ml and 125mg/ml against *Alternaria sp*. (Table 4).

Table 1: Antibacterial activity of the aqueous extract of *Cassia alata* leaf showing the zones of inhibition (mm)

TEST ORGANISMS	CONCENTRATIONS		
	1000mg/ml	500mg/ml	250mg/ml
Klebsiella pneumionae	25mm	21mm	17mm
Proteus vulgaris	23mm	20mm	Nil
Escherichia coli	23mm	16mm	Nil
Pseudomonas aeruginosa	21mm	17mm	Nil
Stahylococcus aureus	17mm	14mm	Nil

Nil represents No zone of growth inhibition
Mm represents millimeter

Table 2: Antibacterial activity of the ethanol extract of *Cassia alata* leaf showing the zones of inhibition (mm)

TEST ORGANISMS	CONCENTRATIONS		
	1000mg/ml	500mg/ml	250mg/ml
Klebsiella pneumionae	24mm	22mm	Nil
Proteus vulgaris	25mm	24mm	22mm
Escherichia coli	Nil	Nil	Nil
Pseudomonas aeruginosa	23mm	18mm	Nil
Stahylococcus aureus	24mm	20mm	Nil

Nil represents No zone of growth inhibition
Mm represents millimeter

Table 3: Antifungal activity of the aqueous extract of *Cassia alata* leaf showing the zones of inhibition (mm)

TEST ORGANISMS	CONCENTRATIONS			
	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
Trichonphyton sp.	Nil	Nil	Nil	Nil
Aspergillus sp.	Nil	Nil	Nil	Nil
Alternaria sp.	Nil	Nil	Nil	Nil

Nil represents No zone of growth inhibition
mm represents millimeter

Table 4: Antifungal activity of the ethanol extract of *Cassia alata* leaf showing the zones of inhibition (mm)

TEST ORGANISMS	CONCENTRATIONS			
	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
Trichonphyton sp.	25mm	17mm	Nil	Nil
Alternaria sp.	18mm	16mm	15mm	13mm
Aspergillus sp.	Nil	Nil	Nil	Nil

Nil represents No zone of growth inhibition
Mm represents millimeter

N.B: Using values as indicated by Johnson and Case (2013) based on diameter of zone of inhibition (mm), the results obtained can be interpreted as;

Resistant: 10mm and below
Intermediate: 11mm – 15mm
Susceptible: 16mm and above

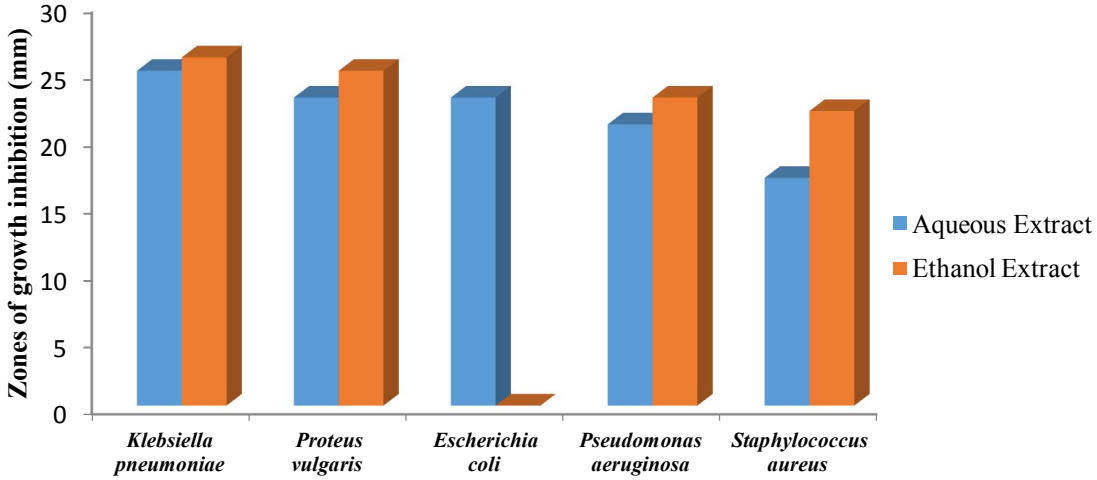

Fig 2: Bar chart showing the comparison between the activities of aqueous and ethanol extracts of *Cassia alata* leaf at 1000mg/ml on the bacteria isolates

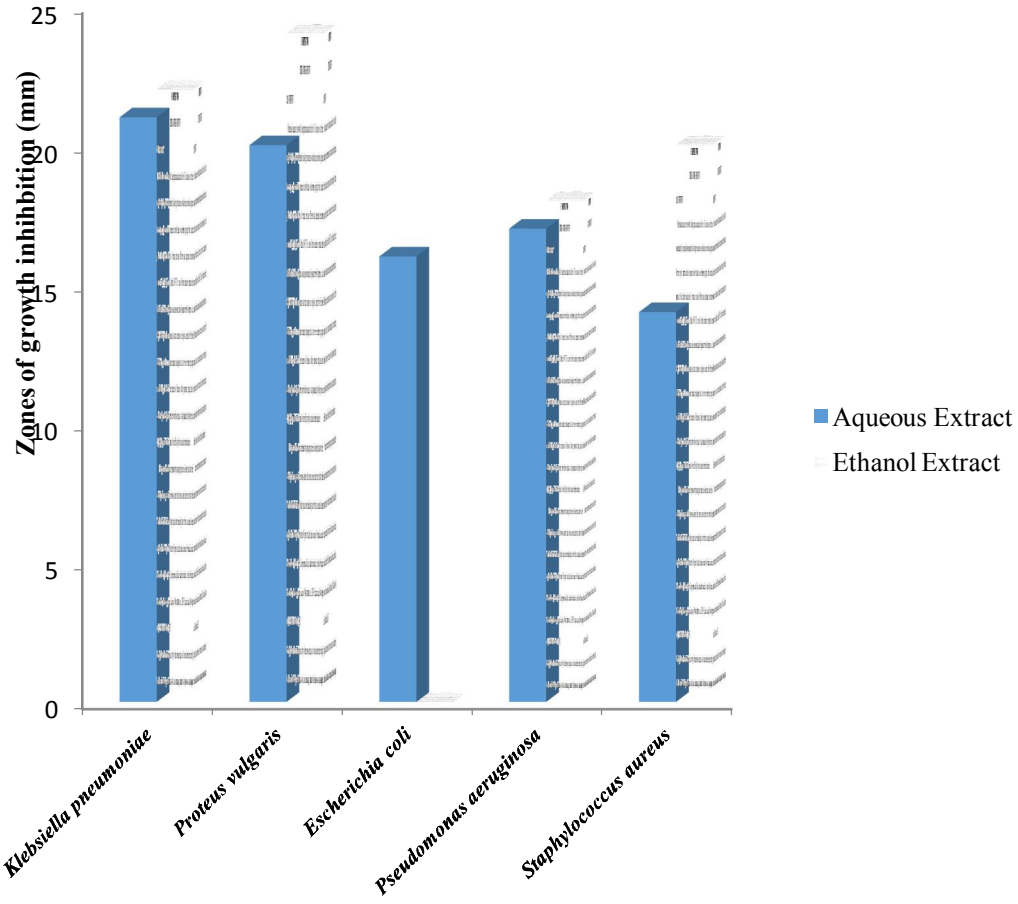

Fig 3: Bar chart showing the comparison between the activities of the aqueous and ethanol extracts of *Cassia alata* leaf at 500mg/ml on the bacteria isolates.

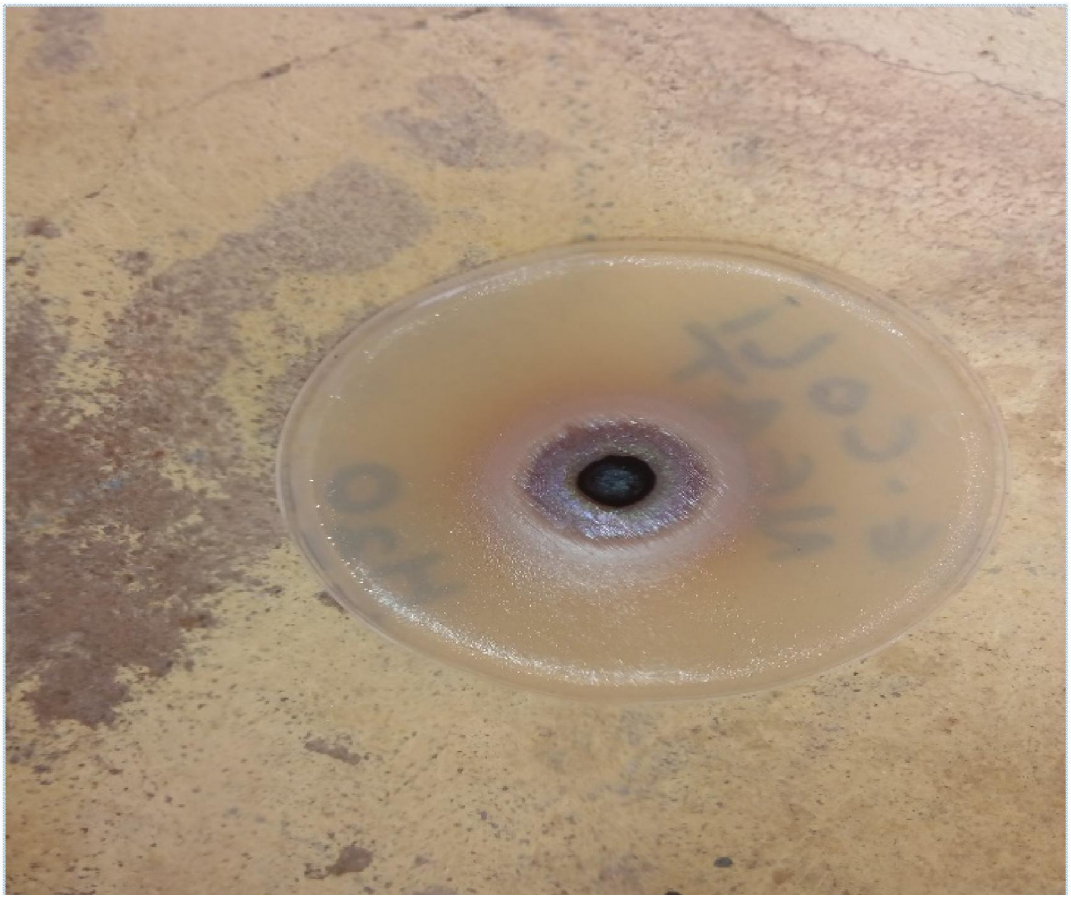

Fig 4: Showing an agar plate with zone of growth inhibition on *Escherichia coli* at 1000mg/ml concentration of aqueous extract of *Cassia alata* leaf

Discussion

The extracts of *Cassia alata* contain different secondary metabolites that have antimicrobial activity (Idu *et al.*, 2007; Odunbaku and Lusanya 2011). These secondary metabolites are well-known to exhibit a variety of biological activity such as antibacterial, antifungal, antitumor, antioxidant, cytotoxic and hypoglycemic activity (Alalor *et al*., 2012). The antimicrobial activity of *Cassia alata* may be attributed to the presence of these metabolites.

It was observed that the ethanol and aqueous extracts of *Cassia alata* leaf demonstrated varying degrees of antimicrobial activity on some of the test organisms at different concentrations (Johnson and Case 2013). The result obtained was similar to the report presented by Timothy *et al*., (2012), as only the aqueous extract of *Cassia alata* leaf showed activity on *Escherichia coli.* There must be some components of the plant that were only water soluble and not soluble in alcohol and so could not be available in the alcohol extract. These components in aqueous extracts that exacted antibacterial activity on *Escherichia coli* could be a topic for further studies. However, contrary to the report of Okoko, (2011), both the aqueous and ethanol extract showed activity on *Klebsiella pneumionae* with the aqueous extract showing higher activity as shown in fig. 2 & 3. ($P<0.05$).

In line with the report of Timothy *et al*., (2012) and Okoko, (2011), the ethanol extract showed higher activity on *Pseudomonas aeruginosa* and *Staphylococcus aereus* than the aqueous extract, ($p<0.05$) but contrary to the report of Timothy *et al*., (2012) the aqueous and ethanol extract showed activity on *Proteus vulgaris,* with the ethanol extract showing higher activity, (fig. 2 & 3). ($p<0.05$).The observed differences in the antimicrobial results obtained could be explained by the different extraction

methodology and extract concentrations of reports in the literature..

The activity of the extracts against the Gram negative bacteria *(Klebsiella pneumionea, Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli*) is impressive because Gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe *et al.,* 2005).

The ethanol extract however, proved to be a better antifungal agent than the aqueous extract as it was observed that there was no antimicrobial activity on any of the fungal species at all the aqueous concentrations used, (Table 3). It is therefore recommended to do further studies on the ethanol extract to determine the components of the plant which conferred it the antifungal property. However, in line with the report of Owoyale *et al.,* (2005) and Somchit *et al.,* (2003) there was activity on Trichophyton sp. and Alternaria sp. but not on Aspergillus sp. as shown in (Table 4). However, Aspergillus species are usually seen as contaminants in foods and superficial or skin clinical specimens. It, however, can be regarded a serious pathogen if found in sterile tissues like the blood, lungs and the endocardium. Aspergillus species can be found as contaminants in soybean products, Mordi *et al.,* (2016).

The antifungal property of the ethanol extract is a welcome development since many fungi especially the dermatophytes, have developed resistance to the available antifungal agents in the market. One of the fungal test organisms was a multidrug resistant Trichophyton isolate from a clinical specimen from a Tertiary Teaching Hospital in Benin city, Nigeria. This Trichophyton species was quite susceptible to the ethanol extract. The formulation of a drug which includes *Cassia alata* as an active component either in the form of cream for topical use or oral for systemic use will be a welcome development.

Using standard values as indicated by Johnson and Case (2013) based on diameter of zone of growth inhibition (mm), the result obtained can be interpreted thus; *Klebsiella pneumionae* was the most susceptible to the aqueous extract followed by *Proteus vulgaris, Escherichia coli, and Pseudomonas aeruginosa* while the least susceptible was *Staphylococcus aureus*. For the ethanol extract, *Klebsiella pneumionae* was the most susceptible followed by *Proteus vulgaris* and *Pseudomonas aeruginosa* while the least susceptible was *Staphylococcus aureus*. *Escherichia coli* was resistant to all the concentrations considered. Of all the fungal species tested, the most susceptible was *Trichonphyton sp.* while the least susceptible was *Alternarias sp.* and the *Aspergillus sp.* was resistant to all the concentrations considered.

The inhibiting activities exhibited by the extracts on the test organisms tend to agree with the reports in the literature (Timothy *et al*., 2012; Moriyama *et al*., 2001; Idu *et al*., 2007; Odunbaku and Lusanya, 2001 and El-mohmood and Doughari, 2008). These literature reports linked antimicrobial activities of *Cassia alata* to the presence of bioactive compounds. The differences in polarity among the various solvents of the aqueous and ethanol extracts are perhaps responsible for the differences in solubility of plant boiactive compounds, hence variation in degree of activity (Srinivasan *et al.,* 2001), as shown in figure 2 & 3.

Escherichia coli was resistant to the ethanol extract, indicating some intrinsic factor in the organism that conferred resistance to the extract. The result of the present study signifies the potential of *Cassia alata* as a source of therapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants. Further studies are recommended to determine the phyto-constituents and purification of individual groups of bioactive components which can reveal the exact potential of the plant to inhibit several pathogenic microbes for the benefit of mankind. The antimicrobial effect of this plant is a welcome development especially its effect on Staphylococcus which is pre-eminent in most skin lesions (Tong et al 2015; Mordi and Ibadin, 2010). Formulations containing extracts of Cassia alata have proved to be effective (Stephen and Akaimo 2014). The susceptibility of Pseudomonas species to the extracts is encouraging. Pseudomonas species are known to be resistant to antibiotics, and some strains are multiple drug resistant (Odjadjare et al., 2012).

Conclusion:

The antimicrobial activity and other health benefits associated with this plant has made it one of the most useful plants to mankind. Further studies are recommended to know more about this plant and determine how to tap all the health benefits which are found in this plant. The use of the extract is therefore encouraged to complement the orthodox medication now available in our markets.

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