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(RESEARCH ARTICLE)



## Chemical analysis and antibacterial activities of *Calotropis procera* and *Clusia rosea* leaves extracts

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### Abstract

In this work, aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves were evaluated for phytochemicals and antibacterial activities in an attempt to evaluate their medicinal potentials. The results of the phytochemical screening showed the presence of alkaloids, saponins, glycosides, tannins, and phenolic compounds in the aqueous extracts of *Calotropis procera* (AqCp) and *Clusia rosea* (AqCr), and ethanolic extracts of *Calotropis procera* (EtCp) and *Clusia rosea* (EtCr). The concentrations of the bioactive constituents in the extracts were in order AqCp > EtCp > AqCr > EtCr. The antibacterial activities were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pyrogenes* with different concentration of the plant extracts. The results of the antibacterial evaluations indicate the plant extracts can be used to inhibit the growth of the bacteria with AqCp being more effective. The result of this study validates the use of the aqueous extract of plant extracts in ethnomedicine and this could provide a lead in the isolation of antibacterial agents from water extracts of medicinal plants.

**Keywords:** *Calotropis procera*; *Clusia rosea*; Antibacterial activities; Ethnomedicine

### 1. Introduction

For years, nature has been the source of several invaluable medicines and medicinal agents. Medicinal plants are the gifts of nature that are widely employed to treat several ailments. The medicinal properties of these plants are due to the presence of secondary metabolites that produce definite physiological action on the human body [1]. Plants are endowed with the natural ability to synthesize and store a great diversity of bioactive substances [2 – 4] with therapeutic tendencies. Some of these metabolites have been successfully isolated and used in the treatment and prevention of diseases [5]. Among the most essential plants' bioactive chemical components are alkaloids, flavonoids, tannins, terpenoids and phenolic compounds [6]. The roles played by plant bioactive substituents in disease control have been demonstrated by many researchers in recent years.

*Calotropis procera* belongs to the Asclepiadaceae family. It is popularly known as the desert wick, *calotrope*, *calotropis*, dead Sea fruit, giant milk weed in English; *bomu bomu* in Yoruba; *tumfafia* in Hausa; *Oshar* in Sudan; *madar*, *akada*, *akdo*, *aak* in Hindi; *bomba*, *algodón extranjero* in Spanish; *mpamba* in Swahili; *ipekag* in Turkish; *aalaka* in Sanskrit; *boah* in Somali; *wahre mudarppflanzer*, *gomeiner* in German, *calotropo* in Italian, *pomme de sode* in French, *mandara* in Marathi and *kisher*, *debaj* or *usher* in Arabic [7 – 11]. It is a xerophytic, erect shrub, growing widely throughout the tropical and sub-tropical regions of Asia and Africa [11]. *Calotropis procera* has considerable potential as a potent medicine in the treatment of leucoderma, leprosy, ulcers, tumors, and piles, diseases of the spleen, liver and abdomen [12, 13]. The pharmacological effects such as antidiabetic, antimicrobial, anti-inflammatory, renal protective, analgesic and anticancer, anti-angiogenic, immunological, antioxidant, cardiovascular, hepatic protective, antidiarrheal, anticonvulsant, antifertility and enhancement of wound healing by *Calotropis procera* have been reported [14, 12].

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*Clusia rosea* belongs to the family of Clusiaceae [15]. *Clusia rosea*, also known as an autograph tree, balsam apple, copey, and pitch-apple, is a tropical and sub-tropical evergreen plant species that can grow up to 18 metres tall [16]. The tree is commonly found in the Caribbean. It is cultivated as an ornamental and street tree. The tree has many local uses and often harvested from the wild for its wood, latex and medicinal applications [17]. The resinous latex obtained from the plant is used medicinally and as a plaster.

Furthermore, members of the *Clusia* family had shown to have an inhibitory effect on human immunodeficiency virus (HIV) [18 – 20]. Leaves of *Clusia sp.* Are often used to soften the skin and have a potential benefit in skincare [21]. This work sought to investigate the chemical composition, and antibacterial activities of *Calotropis procera* and *Clusia rosea* leaves extracts.

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## 2. Material and methods

### 2.1. Plant sample collection and preparation

Fresh leaves of *Calotropis procera* plant and *Clusia rosea* were collected from Odo Ado and Government reserve areas of Ado-Ekiti. Both plants were identified and authenticated in the plant science laboratory, Federal Polytechnic, Ado-Ekiti. The leaves were moved to the laboratory, washed under running water and dried under shield for 10 days. The dried leaf was pulverized with blender, sieved and stored in polythene bags for further analysis. Each plant leaf powdered was extracted separately with water and 95% ethanol. The extraction was done by soaking 20 g of each leaf powder in a beaker containing 200 ml of a specific solvent for 48 hours with periodic agitation. After 48 hours, the content of the beaker was filtered using Whitman No. 1 filter paper and the filtrates were concentrated in a water bath at 65 °C. The extracts were stored in different airtight containers, labelled and kept at 4 °C until further analysis.

### 2.2. Phytochemical Screening

The phytochemical screening of aqueous and ethanolic extracts of both *Calotropis procera* and *Clusia rosea* leaves was done using the methods reported by Abegunde [22].

### 2.3. Test organisms

The microorganisms used for the antibacterial activities are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and *Streptococcus pyrogenes*

### 2.4. Antibacterial assay

The antibacterial assay was performed using a method reported by Bilal *et al.*, [23]. Nutrient agar media used for antibacterial assay were obtained from the microbiology laboratory, Federal polytechnic, Ado-Ekiti. The Agar media were prepared and sterilized at 121 °C for 20 minutes in an autoclave. The nutrient agar media were transferred into a separate sterilized petri dish in laminar air flow, allowed it to cool and then solidified. Bacterial culture was inoculated through the cotton swab method. After the inoculation and uniform distribution of bacteria on the surface of the media, prepared discs with different plant extract concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) were impregnated on the surface of the media using forceps and the Petri dishes were transferred to an incubator for incubation. The incubation was done at 37 °C for 24 hours and the zone of inhibition was measured using a transparent meter ruler and recorded.

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## 3. Results and discussion

### 3.1. Phytochemical screening results

The results of the phytochemical examinations of aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves are presented in Table 1. The availability of the bioactive materials in plant extracts depends on the solvent used. This is because of the degree of solubility of the bioactive constituents in a different solvent. The results revealed the presence of alkaloids, saponins, glycosides, tannins and phenolic compounds in the aqueous and ethanolic extracts of both plants. Terpenoids were present in the extracts of *Calotropis procera* and its aqueous extract revealed the presence of flavonoids. However, both flavonoids and terpenoids were absent in the extracts of *Clusia rosea* using alkaline reagent test and Salkowki's test respectively. From Table 1, the aqueous solvent can be said to be more effective for the extraction of bioactive materials. This result was similar to the report of Abegunde and Ayodele-Oduola [11]. Tannin-rich plant extracts are known to exhibit strong antimicrobial activity by preventing the much-needed protein from being available for the organism and facilitate the precipitation of microbial protein [24]. Furthermore, saponin has been reported with expectorant activity on both gram-positive and gram-negative bacteria [6, 25, 26].

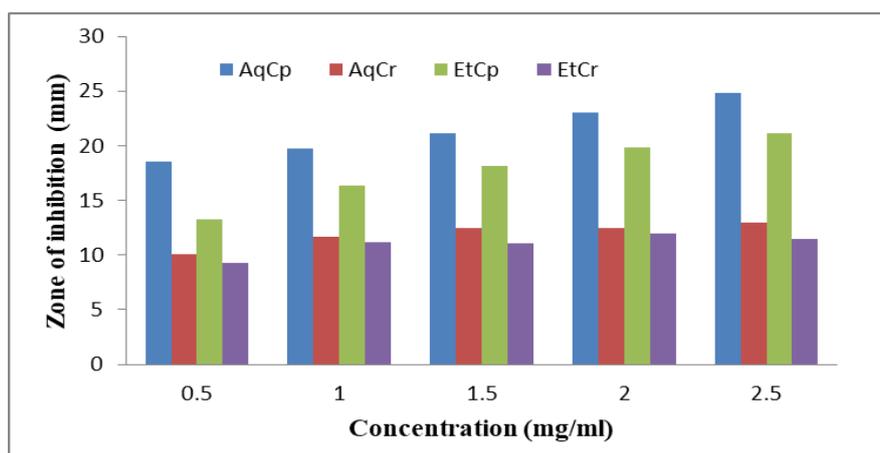
**Table 1** Result of phytochemical screening of aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves.

Plant	Solvent	Alkaloids	Saponins	Flavonoids	Terpenoids	Glycosides	Tannins	Phenolic Compounds
		Wagner's test	Foam test	Alkaline reagent test	Salkowki's test	Keller Kelliani's test	Braymer's test	Ferric chloride test
<i>Calotropis procera</i>	Aqueous	++	++	++	++	++	++	+
	Ethanol	++	++	-	++	++	++	+
<i>Clusia rosea</i>	Aqueous	++	+	-	-	++	+	+
	Ethanol	++	++	-	-	+	+	+

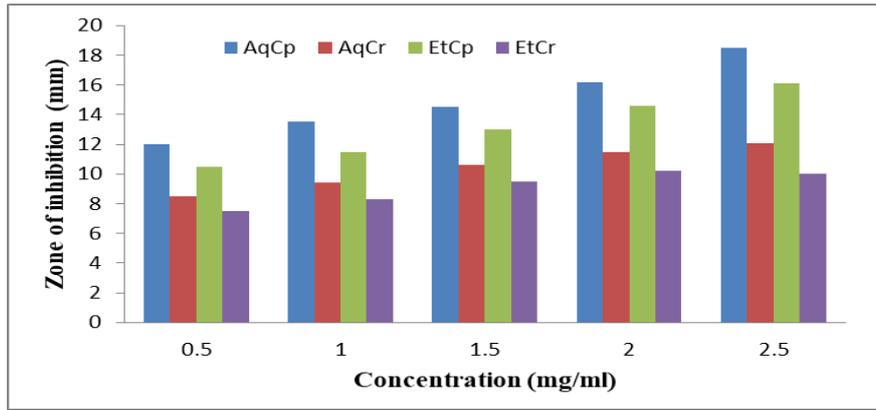
++= present in higher concentration, += present in moderate concentration, -=absent (comparison was made along each column)

### 3.2. Antibacterial activity results

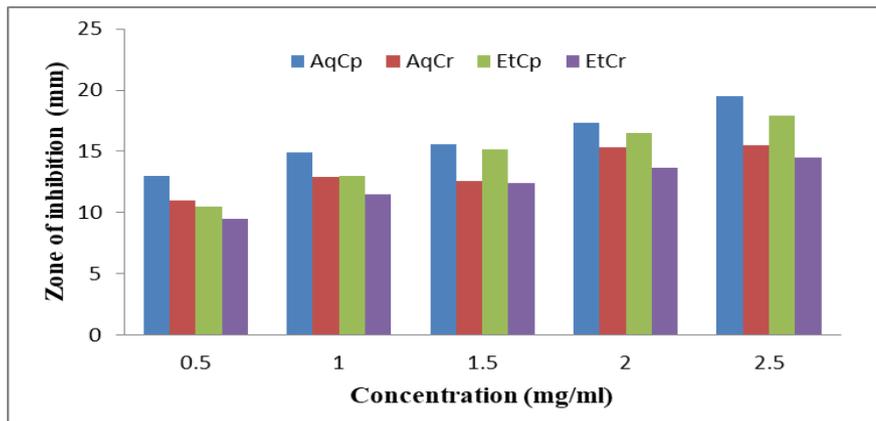
The results of the antibacterial activity of the aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves were measured in terms of the diameter of the zone of inhibition and presented in Figure 1 – 5. Figures 1, 2, 3, 4 and 5 represent the performances of the aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves to inhibit the development of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pyrogenes* respectively at different concentrations. The results revealed that the extracts are effective as inhibition agents against the bacteria. This result supported earlier report by Farooq *et al.*, [27] that extracts of plants are potent against some bacteria. However, the performances increased with an increase in the concentration of each extract. Generally, the aqueous extract of *Calotropis procera* was more effective against all bacteria while ethanolic extract of *Clusia rosea* showed the least potency against the bacteria at each concentration dose. The result of the present work justifies the use of an aqueous extract of *C. procera* in ethnomedicine for the treatment of infectious diseases caused by bacteria [28]. The highest performance of  $24.80 \pm 20$  was recorded for 2.5 mg/ml aqueous extract of *Calotropis procera* against *Escherichia coli*. *Escherichia coli* showed the most susceptibility to the plant extracts while *Salmonella typhi* showed the least susceptibility to the plant extracts.



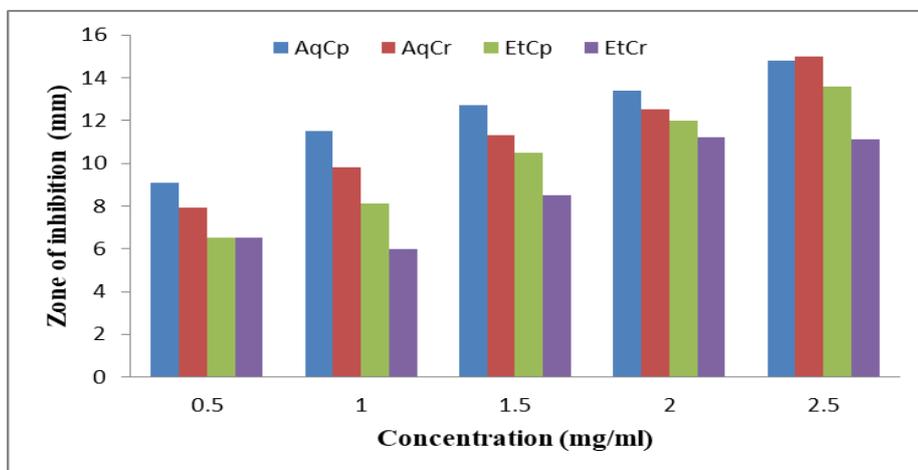
**Figure 1** Diameter of the inhibition zone on the growth of *Escherichia coli* against aqueous and ethanolic leaves extracts of *Calotropis procera* and *Clusia rosea* at different concentration



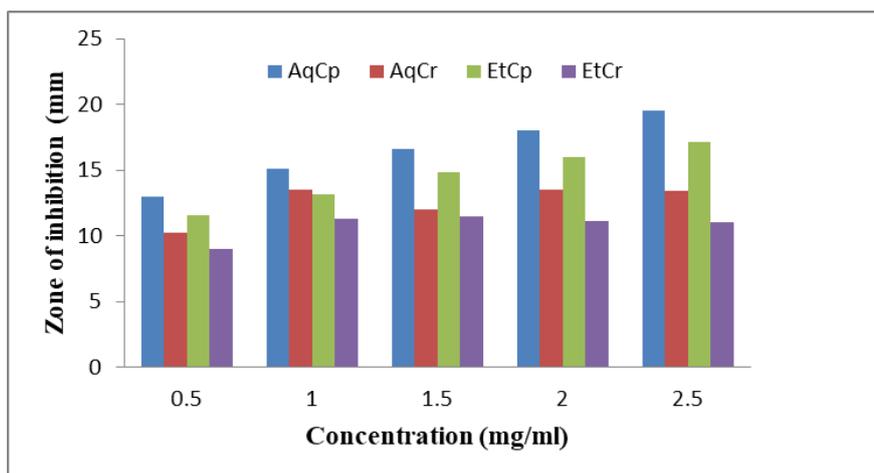
**Figure 2** Diameter of the inhibition zone on the growth of *P. aeruginosa* against aqueous and ethanolic leaves extracts of *Calotropis procera* and *Clusia rosea* at different concentration



**Figure 3** Diameter of the inhibition zone on the growth of *S. aureus* against aqueous and ethanolic leaves extracts of *Calotropis procera* and *Clusia rosea* at different concentration



**Figure 4** Diameter of the inhibition zone on the growth of *Salmonella typhi* against aqueous and ethanolic leaves extracts of *Calotropis procera* and *Clusia rosea* at different concentration



**Figure 5** Diameter of the inhibition zone on the growth of *S. pyogenes* against aqueous and ethanolic leaves extracts of *Calotropis procera* and *Clusia rosea* at different concentration

#### 4. Conclusion

The successful phytochemical screening of aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* revealed the presence of some bioactive constituents. The results of the antimicrobial activities have shown that aqueous and ethanolic extracts of *Calotropis procera* and *Clusia rosea* leaves inhibit the growth of some bacteria. The inhibitory tendency increased with increase extract dosage. It could, therefore, be concluded that the extracts of both plants can be used to retard the growth of some bacteria.

#### Compliance with ethical standards

##### Acknowledgments

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##### Disclosure of conflict of interest

There is no conflict of interest in publishing the present data of the study.

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