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Evaluation of Antimicrobial Activity of Calotropis Species

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Abstract

Calotropis (Asclepiadaceae) often known as "madar" is an important medicinal plant. The current investigation includes the Phytochemical and Antimicrobial study of Calotropis species. Solvent such as methanol, butanol, acetone, pet ether, chloroform and water were used. The antimicrobial study executed by agar well diffusion technique.

Alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids are found in both plants according to phytochemical study. For antibacterial investigation Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa were used. The susceptibility of bacterial strains against different solvent extracts was tested using the agar well diffusion method.

Butanol and methanol extracts have better antibacterial activity than other extract because their zones of inhibition are larger.

Keywords: Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Agar well diffusion method, etc.

Introduction

Nature has provided medicinal substances for thousands of years, and an incredible number of modern medications have come from natural sources, primarily based on their traditional medicine. Various studies have shown that plants are a significant source of medication discovery and development [1]. Calotropis species is a medicinal plant in the family Asclepiadaceae that has been used to treat ailment in Ayurveda, Unani, Sidha and many other traditional systems [2]. Plants have been used to treat a several diseases since ancient times. Arka species (Calotropis gigantean and calotropis procera) are important in Ayurveda because they can be used interchangeably and have similar benefits [3]. In ancient Ayurvedic medicine, Calotropis procera was known as "Shweta Arka", whereas Calotropis gigantean was known as "Shyama arka". Both the species are typically botanically similar and have similar pharmacological properties [4]. Calotropis species are drought-resistant, drought-tolerant and salt -tolerant [2].

Calotropis has been used for variety of puposes since ancient time. Tannin utilised as dystuff, and an acerated bark extract can be used for de- hairing hides and tanning. The bark and latex used as arrow and spear poison. Calotropin, the primary component in latex, is cardio toxic [5].

Figure No.1: Calotropis gigantea

Figure No.2: Calotropis procera

1.2.4 Scientific Classification

Kingdom - Plantae Division -Dicotyledonae Class - Magnoliopsida Order –Gentianales Genus - Calotropis R. Br.

Species – Calotropis procera (Ait) R. Br.

Species - Calotropis gigantean R. Br.

Therapeutic uses of Calotropis gigantea: The whole plant has tonic, anthelmentic and expectorant properties when dried. The roots also have similar properties and can be used as laxative. The powdered root has traditionally been used to treat bronchitis, asthma, leprosy, eczema and elephantiasis, while the latex has traditionally been used to treat vertigo, baldness, hair loss, toothache, intermittent fevers, rheumatoid/joint swellings, and paralysis, the leaves have traditionally been used to treat joint pain and edoema [6].Calotropis gigantea has been scientifically proven to have anti-Candida activity, cytotoxic activity, antipyretic activity and wound healing activity[7].

Therapeutic uses of Calotropis procera: Calotropis procera is used for diarrhea, sinus fistula, and skin diseases. Snake bite, sinus fistula, rheumatism, mumps, burn injuries and body aches were all treated with leaves. These leaves can also be used to treat jaundice[8]. The flower is a tonic that can be used as an appetizer and to relieve stomach aches, as well as to treat piles and asthma. As a toothbrush fresh roots are utilized and are

believed to relieve toothaches. [9]

Chemical constituents of Calotropis gigantea: Calotropin, uscharin, calactin and uscharidin; gigantin are cardiac glycosides found in plant flowers [10]. It also contains the protease calotropin D1 and D2 and calotropin F1 and F2. The flowers contain some toxic elements that have a caustic effect on mucus membrane and sensitive skin, potentially causing secondary dermatitis.

Chemical Constituents of Calotropis procera: The most common cardenolides identified in plant are voruscharin, uscharidin, uzarigenin, calotroposides, calactin, calotoxin, uscharin, ascleposide, calotropagenin, calotropin, proceroside, proceragenin and syriogenin [11].

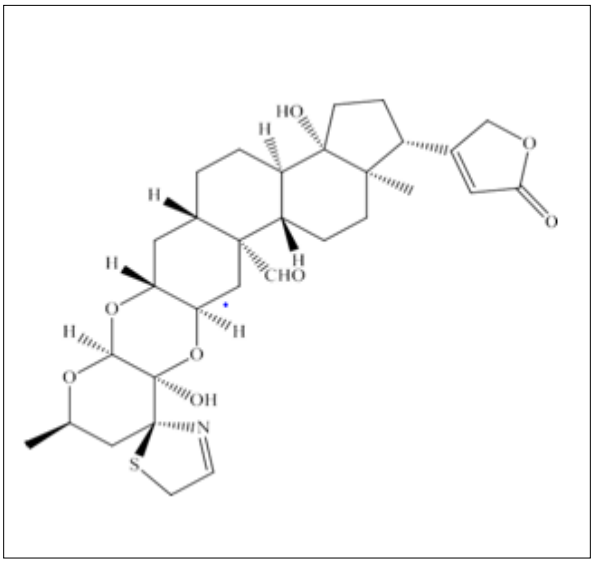


Figure No.3: Uscharin

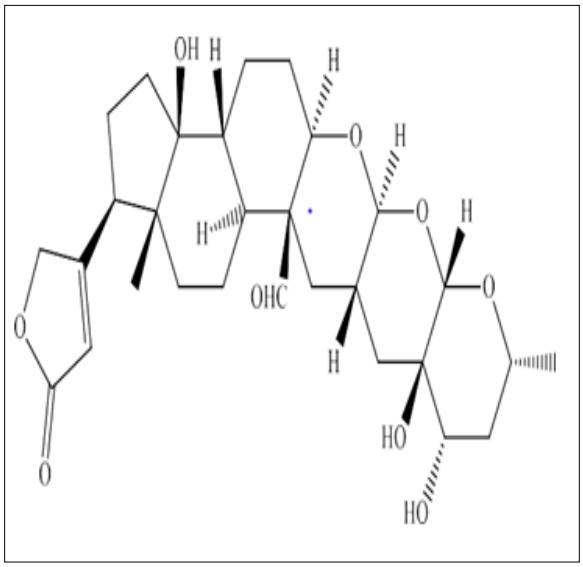


Figure No.4: Calotropin

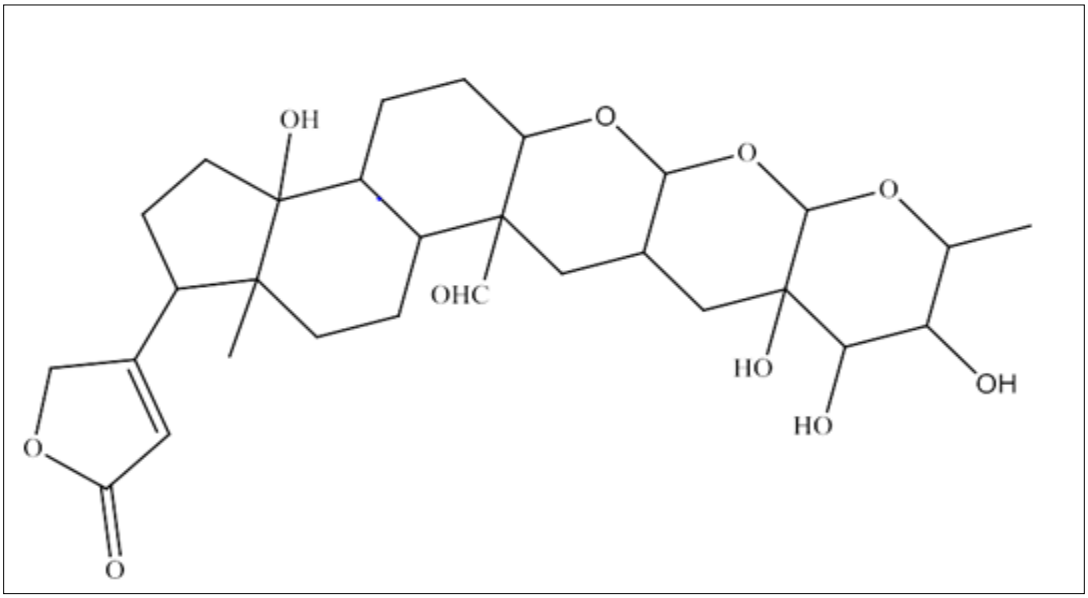


Figure No.5: Calotoxin

Materials and Methods

Chemicals of analytical grade such as butanol, methanol, pet ether procured from High purity laboratory chemicals, Pvt, Ltd. Other chemicals such as acetone, chloroform were obtained from Molychem, Mumbai, Sodium chloride, hydrochloric acid and sulphuric acid from Merck Specialities Pvt. Ltd. Other chemicals were also used such as ethanol, dragendroff's reagent and wagners reagent.

Collection, identification and authentication of plant material

Calotropis gigantea and Calotropis procera plants were obtained from the Rauza bag region of Aurangabad. Both the plants deposited vide accession number 851 and 852 in Herbarium, Late Babruwan Vitthalrao Kale Ayurved Medical College and Hospital, Latur. The fresh flowers from the plant's Calotropis gigantea and Calotropis procera was collected and shade dried prior to reducing it to a fine powder. The powdered material i.e. flowers was ground into fine powder by an electric mill.

Maceration Extraction method

5 gm of air-dried plant material (i.e. flowers) of Calotropis gigantea and Calotropis Procera belonging to the family Asclepiadaceae was macerated separately with 250 ml of Butanol, chloroform, acetone, methanol, ether and water for 72 hours on a rotary shaker. The macerate was filtered and evaporated. The solution obtained was stored at 4^0 C for further studies.

Figure No.6: Maceration of extract

Phytochemical screening

a. Test for alkaloids

A few drops of Wagner's reagent and 2-3 ml of test solution are added. Alkaloids are detected by the presence of Reddish brown coloured precipitates. [12]

b. Test for tannins

In a test tube, about 0.5g of the extract was boiled in 10 ml of and then filtered. A few drops of 0.1% ferric chloride were added and the coloration was examined for brownish green or a blue black. [13]

c. Test for steroids

1.0 ml concentrated sulphuric acid was added to 1.0 ml plant extract, followed by 2.0 ml acetic anhydride solution. The presence of steroids was indicated by a greenish colour that turned blue. [14]

d. Test for flavonoids

A portion of the aqueous filtrate of plant extract was treated with 5 ml of dilute

ammonia solution, followed by addition of concentrated H_2SO_4. The presence of flavonoids was detected by the formation of yellow colour in each extract. [15]

e. Test for Saponins (Foam formation test)

The plant extract was diluted with distilled water and this was shaken for 15 minutes in graduated cylinder. Foam formation indicated the presence of saponins. [16]

f. Test for terpenoids (Salkowwski test)

2 ml of Chloroform and conc. H_2SO_4 were added to 0.5 ml of extract. The presence of terpenoids is indicated by the red-brown colour at the interface. [17]

Table No.1: Preliminary phytochemical determination of Calotropis gigantea

Solvent	Alkaloid	Tannin	Saponin	Phenol	Flavonoid	Terpenoid
Acetone	+ve	-ve	+ve	-ve	+ve	+ve
Aqueous	+ve	-ve	+ve	-ve	-ve	-ve
Chloroform	+ve	-ve	+ve	-ve	-ve	+ve
Pet. Ether	+ve	-ve	+ve	-ve	-ve	+ve
Methanol	-ve	+ve	+ve	+ve	-ve	+ve
Butanol	+ve	+ve	+ve	+ve	+ve	+ve

Table No.2: Preliminary phytochemical determination of Calotropis procera

Solvent	Alkaloid	Tannin	Saponin	Phenol	Flavonoid	Terpenoid
Acetone	+ve	-ve	+ve	-ve	+ve	+ve
Aqueous	+ve	+ve	+ve	-ve	-ve	-ve
Chloroform	+ve	-ve	+ve	-ve	-ve	+ve
Pet. Ether	+ve	-ve	+ve	-ve	-ve	-ve
Methanol	-ve	+ve	+ve	+ve	+ve	+ve
Butanol	+ve	+ve	+ve	+ve	+ve	+ve

*** +ve = present, -ve= absent**

Antimicrobial activity studies

The antibacterial activity of several pathogenic bacterial strains were investigated using agar well diffusion method was carried out.

Preparation of nutrient broth is followed

1. Nutrient agar – 2.8 gm.
2. Distilled water - 100ml
3. PH – 6.8

Bacteria used

1. Pseudomonas aeruginosa (Gram-negative bacteria)
2. Bacillus subtilis (Gram-positive bacteria)
3. Staphylococcus aureus (Gram-positive bacteria)

Agar well diffusion method

The antimicrobial activity of aqueous and solvent extract was measured using agar well diffusion method, According to the National Committee for Clinical Laboratory Standard (NCCLS) with sterile swab soaked with bacterial culture to be tested was spread on a nutrient agar plate. Subsequently, Plant

extract was poured into 8 mm diameter wells in the agar medium and allowed to diffuse for 2 hrs at room temperature. The plates were then incubated at 370 for 24 hrs in an upright position. Wells having the same volume of flower extract of Acetone, chloroform, methanol, butanol, pet ether and water. Streptomycin was used as a standard antibiotic. The diameters of the growth inhibition zones were measured in mm after incubation.

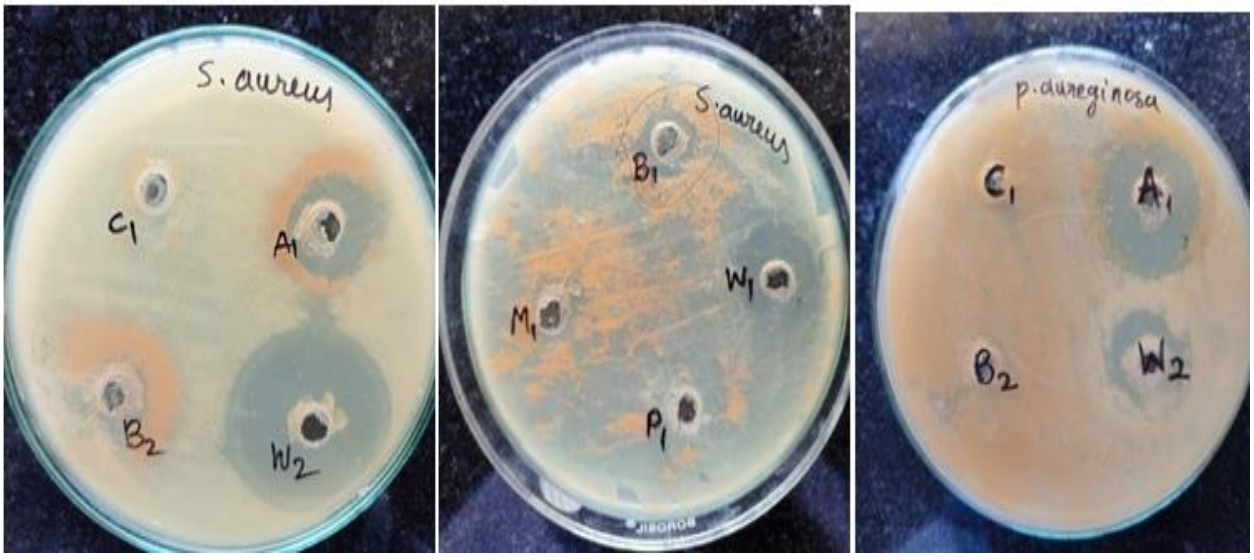
Result and Discussion

The antimicrobial activity of crude extract of Calotropis gigantea and Calotropis Procera (flowers) using methanol, butanol, acetone, pet ether, chloroform, and water was determined. Antibacterial activity of Calotropis species determined against 3 bacteria. Butanol extract of calotropis gigantea show higher zone of inhibition against Gram positive bacteria (staphylococcus aureus) i.e. 28 mm, and Chloroform extract of Calotropis gigantea show no zone of inhibition against staphylococcus aureus, bacillus subtilis and pseudomonas aeruginosa.

Methanol extract of Calotropis procera show higher zone of inhibition against Gram positive bacteria (staphylococcus aureus) i.e. 48 mm and pet ether and chloroform extract of calotropis procera show no zone of inhibition against staphylococcus aureus.

The result obtained confirmed therapeutic potency of Calotropis gigantea and Calotropis procera used in traditional medicine. Moreover, the result shows good antibacterial properties and it supports the use in treatment of various bacterial infections.

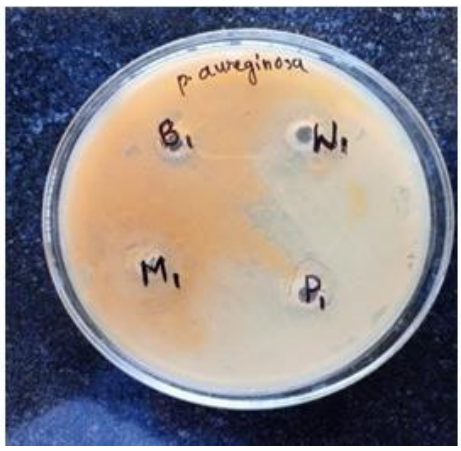
Zone of Inhibition



a **b** **c**


p.aureginosa
M2
P2
C2
A2

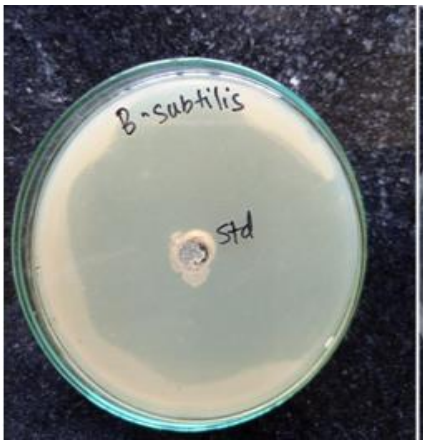
d


p.aureginosa
B1
W1
M1
P1

e


p.aureginosa
B1
W1
M1
P1

f


B.subtilis
Std

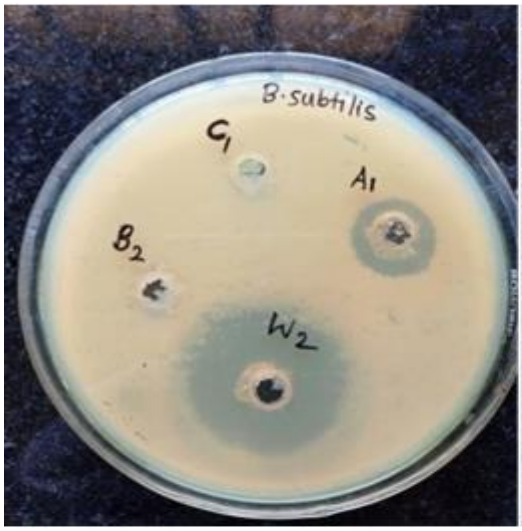
g


B.Subtilis
M2
P2
C2
A2

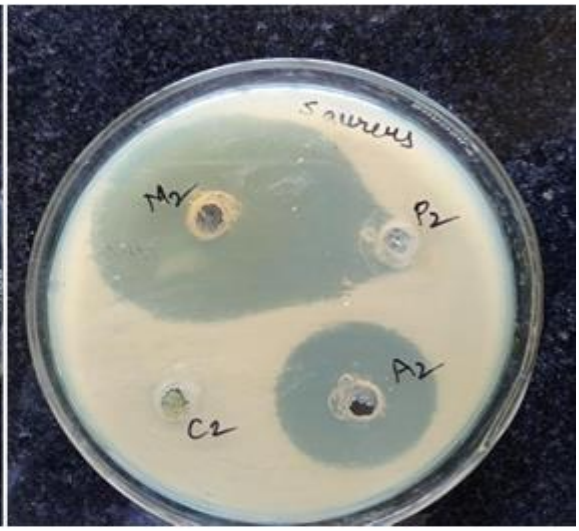
h


B.subtilis
B1
W1
M1
P1

i


B.subtilis
C1
A1
B2
W2

j


A2
P2
A2
C2

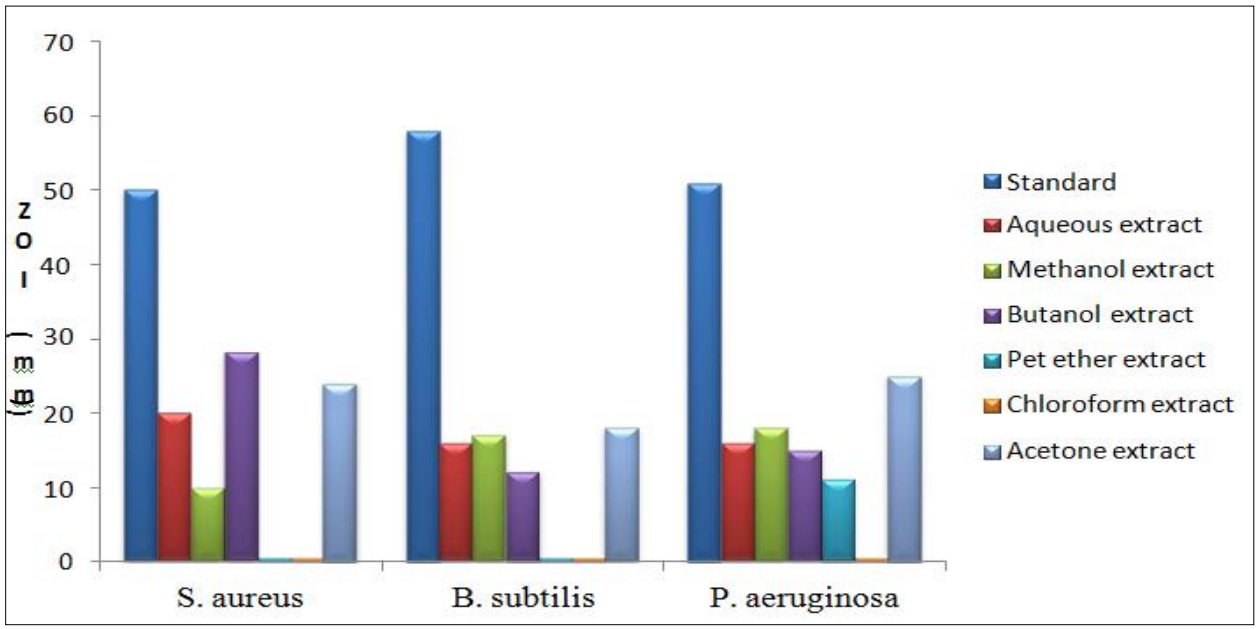
k

[a = S. aureus, b = S. aureus, c = P. aeruginosa, d = P. aeruginosa, e = P. aeruginosa f = P. aeruginosa (Standard),g = B. subtilis (Standard),h = B. subtilis), i = B. subtilis, j = B. subtilis k = S. aureus]

(1 = Calotropis gigantea; 2 = Calotropis procera

M = Methanol, P = Pet ether, C = chloroform, B = butanol, W = Water, M = methanol)

Antibacterial activity of Calotropis gigangtea by agar well diffusion method



Antibacterial activity of Calotropis procera by agar well diffusion method

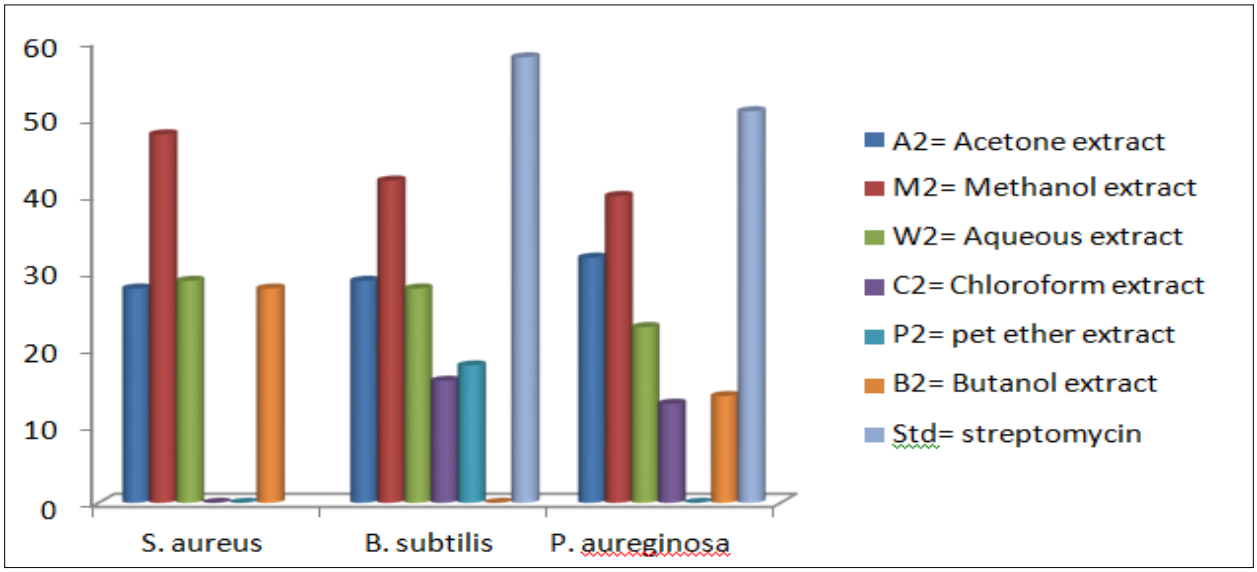


Figure No.7: Graphical representation of zone of inhibition of Calotropis species

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