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Research

Determination Of Combined Antimicrobial Activity Of Stems Of Plants Acalypha Wilkesiana and Cordyline Fructicosa

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Abstract

The study aims to evaluate the combined antimicrobial activity of *Acalypha wilkesiana* and *Cordyline fructicosa* plant stems. Extract is prepared by Maceration process ethanol and water used as solvents. The result obtained for ethanolic and aqueous extract phytochemical screening is performed by various chemicals test as test for Alkaloids, Flavonoids, Tannins, Saponins, Glycosides etc. Followed by antimicrobial activity determined by preparing in combined ratios 2:1, 1:1, 1:2 of two plants Acalypha wilkesiana and Cordyline fructicosa by cup plate method against the test organisms Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli. Results obtained for combined antimicrobial activity as Ethanolic extracts shows maximum activity of 21mm at ratio 1:2 against Staphylococcus aureus and minimum activity of 15mm at ratio2:1against Bacillus subtilis. whereas aqueous extracts shows maximum activity of 20mm at ratio 1:2 against Escherichia coli and minimum activity of 15mm at ratio 2:1against Pseudomonas aeruginosa

Keywords: Wilkesiana, Fructicosa

INTRODUCTION

Plant profile
Acalypha wilkesiana[copperleaf]
*Acalypha wilkesiana*is a fast growing tropical ever green shrub. It is available in different colours likegreen,purple,yellow,orange,copper,pink,white and crimson. (figure 2)

Cordyline fructicosa [Goodluck plant]
Cordyline fructicosa is an evergreen flowering plant in the family Asparagaceae. It is also cultivated for food, traditional medicine, and as an ornamental for its variously colored leaves. It is identified by a wide variety of common names, including ti plant, palm lily, cabbage palm. (figure 1)

Fig: 1 cordyline fructicosa [Goodluck plant]

Fig: 2 Acalypha wilkesiana [Copperleaf]

MATERIALS AND METHODS

Collection of plant material

Cordyline fructicosa (Goodluck plant) stems were collected from Pulla Reddy Institute of Pharmacy, Domadugu (V), SangaReddy (D), Telangana, India.

Acalypha wilkesiana (Copper leaf) stems were collected from PullaReddy Institute of Pharmacy, Domadugu(V), SangaReddy(D), Telangana, India.

CHEMICALS: The chemicals to be used in the present studies are as follows,

- Distilled water
- Ethanol

REQUIREMENTS: Stems of plants Cordyline fructicosa and Acalypha wilkesiana.
Gram positive: Staphylococcus aureus, Bacillus subtilis
Gram Negative: Pseudomonas aeruginosa, E. coli

Instruments

- Macerator, Incubator, Laminar airflow, Autoclave, Incubator, Sterilised inoculating loop, Borer, Spreader.

Experimental methods and apparatus

- Method of extraction used was **Cold Maceration.**
- Various stem extracts to be Analysed and compared for antimicrobial activity.

Sample collection:

- Acalypha wilkesiana and Cordyline fructicosa stems were collected in our college.
- Authenticated by botanist in sultan bazar (koti).
- weeks at room temperature.
- Dried plants materials were powdered finely and stored in sterile bottles to carryout further analysis shown in figure 3 and 4

Dried stem powder of Dried stem powder of Cordyline fructicosa Acalypha wilkesiana

Figure 3

Figure 4

Preparation of Extracts

The powdered samples were extracted using ethanol and aqueous (water) in the ratio of 1:10. The container with its contents were sealed and kept for minimum of 24 hrs (1 day) and maximum of 72 hrs (3 days) under dark condition.

Method of Extraction

MACERATION: The process of extraction in which powdered/size reduced drug is soaked in suitable solvent or mixture of solvents till the solvent penetrates into the drug is called Maceration.The process of maceration is as follows:

Plant material (crushed or cut small or moderately coarse powder

↓

Placed in a closed vessels

↓

Whole of the selected solvent (Menstruum) is added

↓

Allowed to stand for 7 days shaking occasionally

↓

Liquid strained off

↓

Solid residue (marc) pressed (recover as much as occluded solution)

↓

Strained and expressed liquids mixed

↓

Clarified by subsidence and filtration

↓

Evaporation and concentration

Extraction with water and ethanol

- Take 30gms of dried stems of two plants Acalypha wilkesiana,Cordylinefructicosa
- To that add 300ml of water.
- Cover the beaker with the silver foil and shake the beaker continuously at certain interval of time.
- This process is done for 24hrs without any heat (cold maceration).
- Now this is filtered, and the filtrate is collected which is measured.
- We got the filterate of 200ml which is taken in a beaker and covered with the silver foil by making holes on it.
- Now it is kept on a water bath/heating mantle till the filtrate gets viscous.
- Now this viscous filtrate is measured which is about 10ml. use this extract and proceed for phytochemical screening and antimicrobial activity.

❖ aqueous extracts shown in figure 5 and 6 Ethanolic extracts shown in Figure 7 and 8.
❖ 10ml of filtrate for the phytochemical screening.

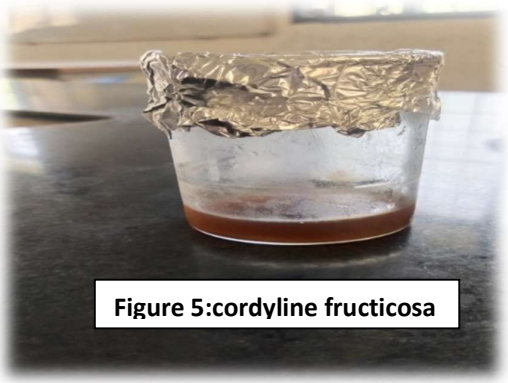


Figure 5:cordyline fructicosa

Figure 6 : Acalypha wilkesiana

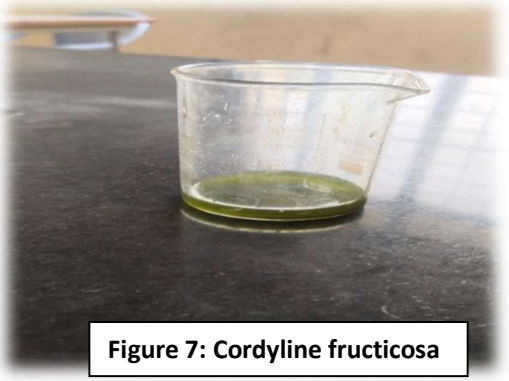


Figure 7: Cordyline fructicosa

Figure 8: acalypha wilkesiana

Plan of work:
Stage 1: Literature review
Stage 2: Extraction of plants using solvents ethanol and water.
Stage 3: Phytochemical screening
Stage 4: Extracts are tested for Antimicrobial studies using different microorganisms.
Stage 5: Evaluation of results and reports of higher activity found for which Plant and extract against the particular organism.

Procedure

❖ **Phytochemical screening:** chemical test were performed for detection of phytochemical chemical constituents as it is shown in table no1.

Table1: Phytochemical Screening

Phytochemical compound	Procedure	Cordyline fructicosa	Acalypha wilkesiana
Alkaloids	2 ml of plant extract + few drops of Hager's reagent	+	+
Flavonoids	2-3 drops pf NaOH+2 ml of plant extract+ few drops of dil.HCl	+	+
Tannins	Few drops of plant extract+ few drops of 2% of $FeCl_3$	+	+
Saponins	1 ml of plant extract is treated with 1% lead acetate solution	+	+
Glycosides	Mix 4ml of glacial acetic acid with 1 drop of 2% $FeCl_3$ + 10 ml of aqueous plant extract + 1ml of conc. H_2SO_4	+	+
Phenols	1 ml of plant extract + few drops of $FeCl_3$	+	-
Terpenoids	5 ml of plant extract + 2 ml of chloroform + 3 ml of conc. H_2SO_4	+	-
Resins	1 ml of plant extract + few drops of acetic anhydride + 1ml of conc.H_2SO_4	-	-
Carbohydrates	Add fee drops of Molisch's reagent to plant extract dissolved in distilled water + 1ml of conc.H_2SO_{4+} dilute with 5 ml of distilled water	-	-
Fixed oils	Add 0.5 N of KOH to the plant extract + add a drop of phenolphthalein + heat on water-bath for 1-2 hrs.	-	-

ANTIMICROBIAL ACTIVITY

Antimicrobial activity: The ability that a drug kills or supresses the growth of microorganisms.Antimicrobials can kill microorganisms and/or prevent their growth by targeting key steps in cellular metabolism such as the synthesis of biological macromolecules, the antimicrobial activity refers to the ability of a substance to inhibit the growth of or kill microorganisms, including bacteria, fungi, viruses, and parasites.

CUP PLATE METHOD

Preparation of Nutrient Agar Plates:

Mixed 2.8 grams of nutrient agar with 100ml of water, then autoclaved at 121°C and 15lbs pressure. Pour onto sterilized Petri plates and let to solidify.

Preparation of Bacterial Culture:

Using sterile disposable loops, inoculated bacterial strains(Staphylococcus aureus and Psudemonous aeruginosa) onto nutrient agar plates. Incubated the plates at 37°C to promote bacterial growth.

Preparation of Test Samples:

Using an ethanol solvent, diluted the test samples to conc. of 0.100 to 1000ug/mL. Prepare a series of dilutions for each testsample

Sterilization of Cork Borer:

With a Bunsen burner, heat the cork borer until it glows red hot. Let the cork borer cool down before using.

Creation of Wells:

Use the sterilized cork borer to make wells in the agar plates for the bacterial lawn. Press the cork borer gently into the agar to make evenly spaced wells.

Addition of Test Samples
Using micropipettes to precisely added small amount of each dilution of the test samplesto the wells. Ensured that each well receive same volume of samples .

Incubation
For 18-24 hours, incubated the plates at 37°C to allow for bacterial growth and test sample diffusion.

Evaluation of Zones of Inhibition
- After incubation, checked the plates for zone of inhibition surrounding the wells.
- Use a ruler or calipers to measure the diameter of any clear zones that have developed around the wells.
- maximum and minimum activities results were shown in figure 9,10,11 and 12

Maximum:

Figure 9: Acalypha wilkesiana ethanol - 24mm

Minimum:

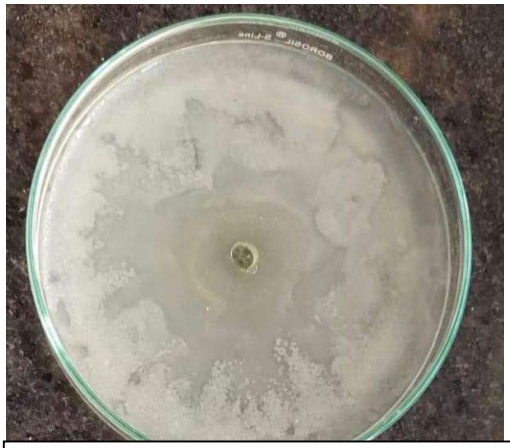


Figure 10: Cordyline fructicosa ethanol -25mm

Figure 11:Acalypha wilkesiana water - 17mm

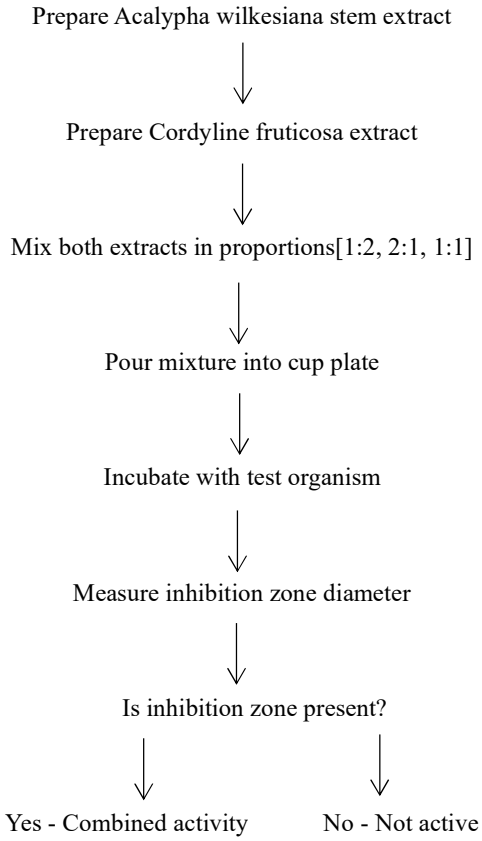
Figure 12: Cordyline fructicosa water - 21 mm

Combined activity
Definition: The cup-plate method is a common technique used in microbiology to test the antimicrobial activity of substances. Here's a procedure for combined activity using the cup-plate method.

Procedure
Different ratios as 1:2, 2:1, 1:1 of plants Acalypha wilkesiana: Cordyline fruticosa are prepared for ethanolic and aqueous extract separately and antimicrobial activity determined using cup plate method and observed for

increased or decreased activity and results were reported results were shown in figure 13,14, 15 and 16 and table 2 and 3.

Flow chart:



Ethanol extract for combined activity:

Figure: 13 Minimum: 15mm

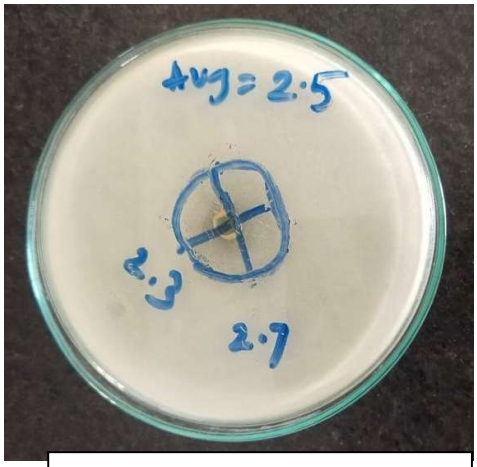


Figure: 14 Maximum: 21mm

Water extract for combined activity

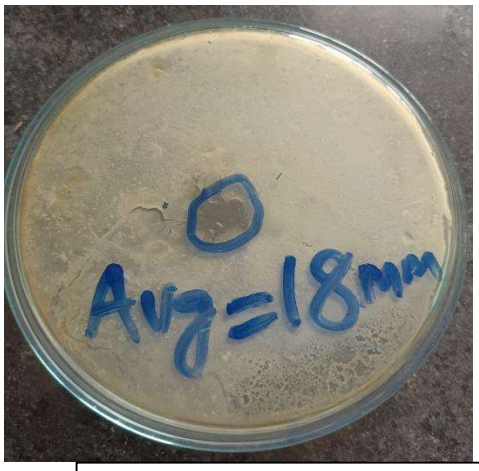


Figure 15: **Maximum: 20mm**

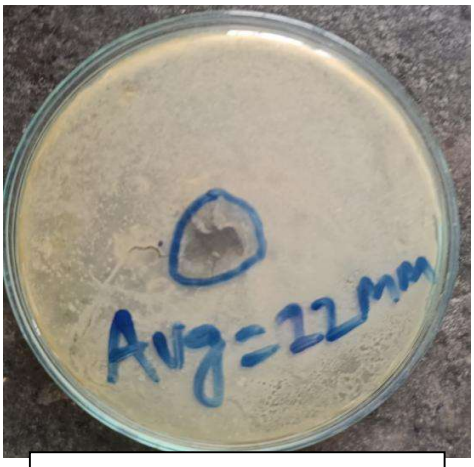


Figure 16: **Minimum: 15mm**

RESULTS

Table 2

Organisms	Acalypha wilkesiana [Ethanol]	Cordyline fructicosa [Ethanol]	Acalypha wilkesiana + Cordyline Fructicosa [Ethanol (2:1)]	Acalypha wilkesiana + Cordyline Fructicosa [Ethanol (1:1)]	Acalypha wilkesiana + Cordyline Fructicosa [Ethanol (1:2)]
Bacillus subtilis	25mm	24mm	15mm	17mm	19mm
Pseudomonas aeruginosa	-	10mm	17mm	16mm	20mm
Staphylococcus aureus	-	-	20mm	18mm	21mm
Escherichia coil	18mm	22mm	18mm	19 mm	20mm

Table 3:

Organisms	Acalypha wilkesiana [Water]	Cordyline fructicosa [Water]	Acalypha wilkesiana + Cordyline fructicosa [Water (2:1)]	Acalypha wilkesiana + Cordyline fructicosa [Water (1:1)]	Acalypha wilkesiana + Cordyline fructicosa [Water (1:2)]
Bacillus subtilis	17 mm	15mm	17mm	17mm	16mm
Pseudomonas aeruginosa	12mm	17mm	15mm	18mm	18mm
Staphylococcus aureus	10mm	18mm	16mm	16mm	19mm
Escherichia coli	16mm	12mm	18mm	19mm	20mm

CONCLUSION

From the present study that is determination of combined antimicrobial activity of stems of plants acalypha wilkesiana and cordyline fructicosa, it is concluded that Ethanolic stem extracts of plant Acalypha wilkesiana shows maximum activity of 25mm whereas Cordyline fructicosa shows maximum activity of 24mm against Bacillus subtilis and combined activity found maximum against against Staphylococcus aureus is 21mm

for ratio 1:2 and minimum activity against Bacillus subtilis is 15mm for ratio2:1. aqueous stem extracts of plant Acalypha wilkesiana shows maximum activity 17mm against Bacillus subtilis whereas Cordyline fructicosa shows maximum activity of 18 mm against Staphylococcus aureus and combined activity found maximum against against Escherichia coli is 20mm for ratio 1:2 and minimum activity against Pseudomonas aeruginosa is 15mm for ratio 2:1.

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