RESEARCH STUDIES ON EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF COLD ETHANOIC EXTRACT OF EUCALYPTUS OIL

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Abstract: Medicinal plants which have antimicrobial compounds act against various pathogens. Myrtle family have different species. Eucalyptus is one among the myrtle family. Eucalyptus have different antimicrobial compounds. The plant grows well in many countries. This fast growing plant have different parts, each part has its own significant medicinal values lik timber, pulpwood, and different essential oils. Our research study showed phytochemical composition and antibacterial activities of eucalyputs leaves. The sample of eucalyptus is collected in the powder form. The eucalyptus powder is mixed into a cold ethanol and dry in a hot air oven. The dried extract was swapped and measured. Weighted 2.096g respectively and then the extract is used for phytochemical analysis and for quantitative analysis and then for antibacterial assay The results of this study suggest that the different concentrations of eucalyptus with various concentrations showed potential antibacterial activity.

***Keywords*: Eucalyptus, pathogenic organism, phytochemical composition, antibacterial activity**

1. Introduction

Fig : Eucalyptus leaves

The Australia originated plant eucalyptus grow in all areas and they are cultivated in different climates. Based on the literature these leaves has essential oils, which showed different antimicrobial activities. Tunisia etal.,1957 introduced nearly 120 species of eucalyptus , used for fire wood and mine wood ,against erosion also. Very less studies reported on this oils and its bio activity at global level. Tunisia *etal* 2010,11 reported first about this oil and its antibacterial activity. Eucalyptus oil showed antiviral activity also., which will useful to treat influenza.Cowam etal 1999studies showed the value of phytomedicins,which will useful to treat different viral infection.

K.D. Hill & L.A.S. Johnson 2012 performed invitro studies of antimicrobial and antioxidant activates of the phenolic extract of eucalyptus leaves.

2. Materials And Methods:

Objectives:

1.sample collection
2.prepration of extraction
3.primary phytochemical analysis
4. sequential extraction
5.antibacterial assay.

3. Sample Collection:

Samples of eucalyptus is collected in the form of powder. eucalyptus was dried in the sunlight until they dry and then grinded into powdered form.

ISSN (Print): 2204-0595
ISSN (Online): 2203-1731

Fig: shows the sample in powder form

SEQUENTIAL EXTRACTION:

The secondary metabolites had extracted from the sample using high polar solvent sequentially. Methanol (C_2H_5OH) is taken as high polar solvent. The sample is kept in a conical flask. And then kept in the hot extraction method. After kept in the hot air oven until it dry. After evaporation the dried extract were scrapped and measured. Then the extract was weighed about 2.096 g respectively. The extract is stored separately.

Extreaction of sample

4. Phytochemical Analysis:

carbohydrates,tannins,saponins,flavonoids, Alkaloids, Quinine, Glycosides, Cardiac glycosides, Terpenoids, Phenols, Coumarins, Steroids and Phytosteroids, Phlobatannins, Terpenoids, Anthraquinones in extract was identified with Indian standard methods.

5. Antibacterial Assay:

The plant pathogenic bacteria is isolated from the spoiled fruit or vegetables and serially diluted and isolated. The isolated bacteria were cultured by mat culturing technique and the wells are punched in agar plates and the plant extract of different concentration was poured in the wells for zone of inhibition.

6. Results And Discussion

Qualitative estimation of primary metabolites. Using standard methods Primary phytochemical analysis of various metabolites was carried out and observed various changes in study sample.

Table: This table showed qualitative estimation of primary metabolites in sample.

TEST	OBSERVATION (COLOUR)	CONFORMATION
Carbohydrates	Brown	Absence
Tannins	Light Black	Absence
Saponins	No foam	Absence
Flavonoids	Yellow	Presence
Quinine	Yellow	Absence
Glycosides	Two Layers	Absence
Cardic Glycosides	Yellow	Absence
Terpenoids	Two White Layers	Absence
Phenols	Bluish Green	Presence
Coumarins	Yellow	Presence
Steroids andPhytosteroids	Two Layers	Absence
Phlobatannins	Yellow	Absence
Anthraquinones	Yellow	Absence

7. Quantitative Analysis:

ESTIMATION OF TOTAL PHENOLIC CONTENT:

Different aliquots [sample and galic acid] (0.2 to 1.0ml) were pipetted into test tubes. 0.5ml of Folin's-Ciocalteau reagent was added, then the test tubes mixed thoroughly after adding with 2ml of 35% of Na2CO3 solution.

Place the tubes in the boiling water bath for exactly one min, cool and measure the absorbance at 650nm against the reagent blank. Prepare a standard curve using different concentrations of catechol.

CONCENTRATION AND ABSORBANCE OF GALIC ACID FOR STANDARD:

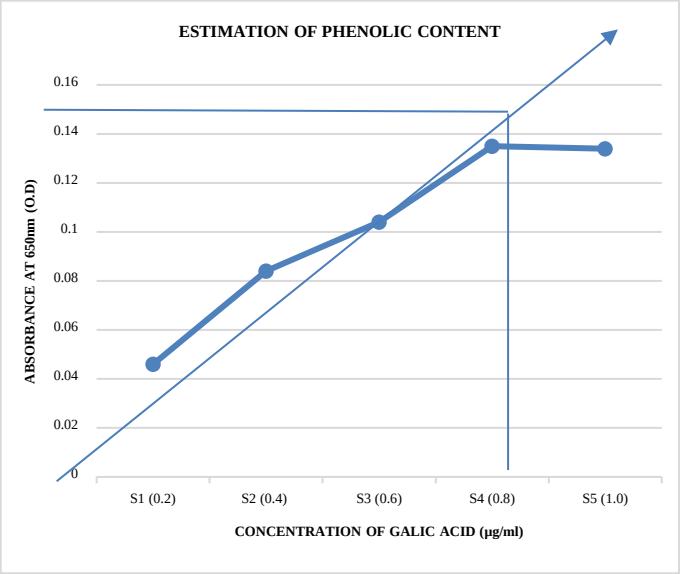
SAMPLE	Corcentration	Absorbance
1	0.2	0.046
2	0.4	0.084

ISSN (Print): 2204-0595
ISSN (Online): 2203-1731

3	0.6	0.104
4	0.8	0.135
5	1.0	0.134

CONCENTRATION AND ABSORBANCE OF EUCALPTUS:

Concentration	Absorbance
0.2	0.149
0.4	0.325



CALCULATION:

0.2 mg/ml of sample contains = 8.6 µg of phenol content

For 0.1 mg of sample = 4.3 µg of phenol

1000 ug of sample contains = 43 µg of phenol.

1mg of eucalyptus contains 43 µg of phenol.

ESTIMATION OF FLAVONOIDS:

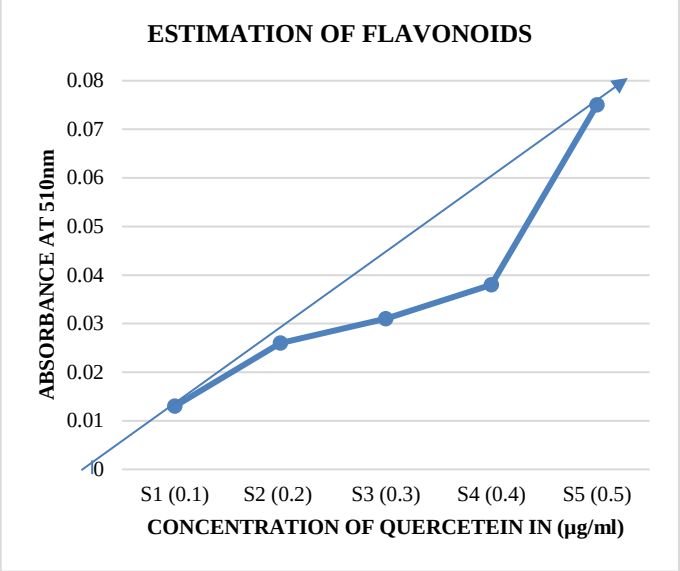
Add 0.5ml sample to test tube containing 1.25 ml of distilled water, 0.075ml 0f 5 % sodium nitrite solution , allowed to stand for 5mins. 0.15ml of 10% aluminum chloride. 6min 0.5ml of 1M NaOH was added and mixture was diluted with another 0.275ml of distilled water. The absorbance of mixture at 510nm was measured immediately (Kim etal.2003).

CONCENTRATION AND ABSORBANCE OF QUERCETIN FOR STANDARD:

Sample	Concentration	Absorbance
1	0.1	0.013
2	0.2	0.026
3	0.3	0.031
4	0.4	0.038
5	0.5	0.075

CONCENTRATION AND ABSORBANCE OF EUCALYPTUS:

Concentration	Absorbance
0.2	0.002
0.4	0.001



Above Graph represented the flavonoids estimation.

CALCULATION:

0.1µg of eucalyptus sample contains 0.2 µg of flavonoids

1 µg = 0.2 µg

1mg = 200µg of flavonoids

1mg of eucalyptus contains 200 µg of flavonoids

ANTIBACTERIAL ASSAY:

Mat culture techniques used to culture the pathogenic bacteria, isolated from spoiled plant materials, and identified antibacterial activity of eucalyptus oil using agar plate method

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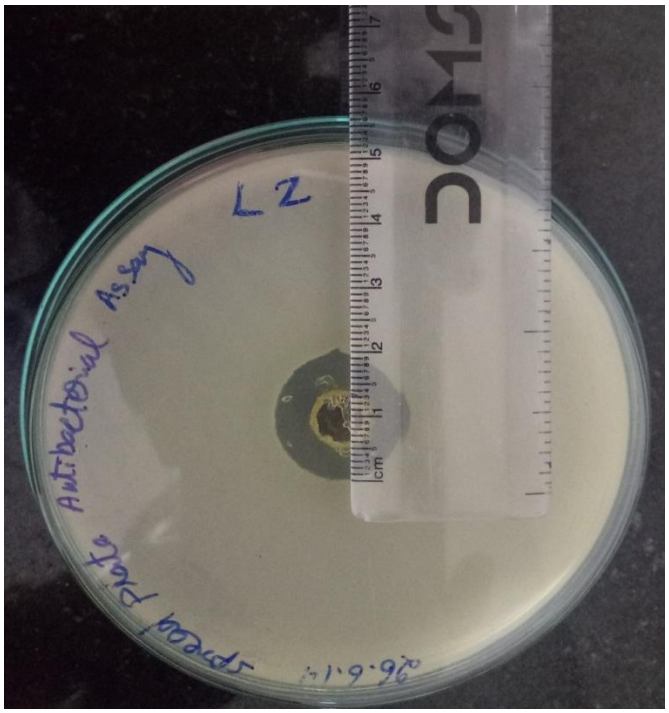
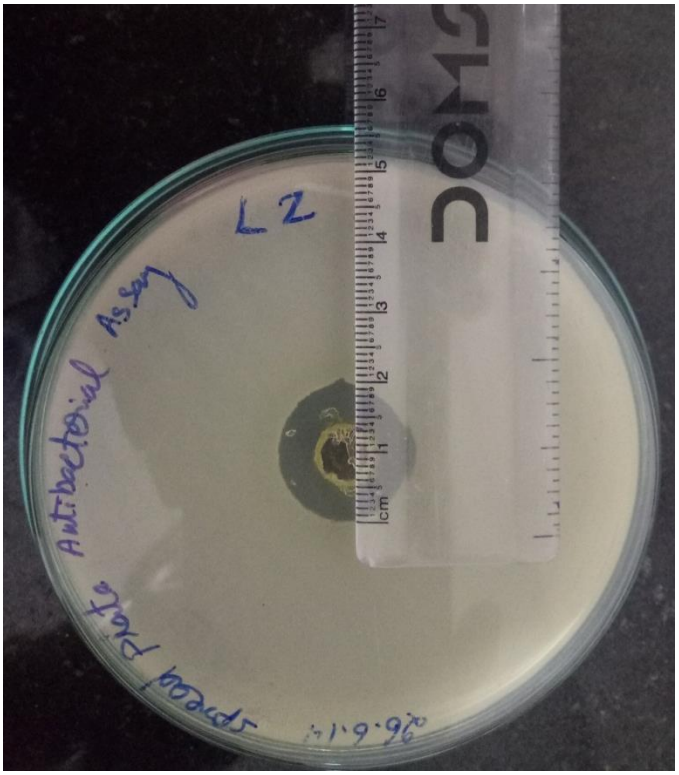


zone of the inhibition of pathogenic bacteria(Pseudomonas)



zone of the inhibition of pathogenic bacteria(Ervinia)

CONCLUSION:

The antibacterial activity of eucalyptus over the bacteria was 20.00 mm average zone of inhibition. This indicates that eucalyptus contains the antibacterial activity. This study also indicates the phytochemical composition and antibacterial activities of eucalyptus. The antibacterial activity of the ethanolic eucalyptus extract had a strong antibacterial activity against bacteria like Pseudomonas and Ervinia.

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ISSN (Print): 2204-0595
ISSN (Online): 2203-1731

ISSN (Print): 2204-0595
ISSN (Online): 2203-1731