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Phytochemical Analysis and Anti Microbial Activity of Cappers Zeylanica Linn

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Abstract

The paper deals with the phytochemical screening and antimicrobial activity of cappers zeylanica linn leaves using different solvents like n-hexane, ethyl acetate, methanol and aqueous. The phytochemical analysis shows the presence of various phyto-constituents like alkaloids, flavonoids, tannins, terpenoids, steroids, carbohydrates, glycosides, etc. The crude extract of different solvents are tested for its antimicrobial activity against Bacillus subtilis, Escherichia coli and Staphylococcus aureus. The maximum activity was shown by methanol extract against Bacillus subtilis which was observed with 20 mm and minimum activity was shown in methanol extract against Staphylococcus aureus observed with 10 mm.

Keywords: cappers zeylanica linn, solvents, extract and antimicrobial activity.

1 Introduction

Capparis zeylanica Linn. is commonly known as Indian caper, a climbing scandant shrub and found throughout India. Capparis zeylanica Linn is belonging to the family Capparidaceae (1,2). These plants are 2-3 m in height, armed with 3-6mm long recurved thorns, branched and the leaves are elliptic or broadly lanceolate (3). Traditionally Capparis zeylania L. was first time reported and was used as vegetable. Its root bark is grounded with water, boiled and taken orally to treat indigestion. Traditionally it is used as an antidote to snake bite, to cure swelling of testicle, small pox, boils, cholera, colic, hemiplagia, neuralgia, sores, pneumonic and pleurisy (4,5). It has been used as a drug in the traditional Ayurvedic system of medicine (6). Capparis species has been reported to have antihelmintic, antimicrobial (Mali et al., 2004) and antiinflammatory (Chaudhary et al., 2004) activities.

2 Experimental Sections

2.1. Collection of plant material

The leaves of cappris zeylanica linn were collected from nearby villages of Tirupattur district, Tamil Nadu and the leaves are washed with water followed by ethanol and dried carefully in the absence of sunlight to remove the water molecules present in the leaves. The dried leaves are made into fine powder using blender. Then the fine powders are stored properly in an airtight container for future purpose.



Fig 2.1 Cappers zeylanica linn leaves

2.2 Preparation of extract

About 40g of the fine powder of the leaves of cappris zeylanica linn are taken in a thimble which is placed in a Soxhlet extractor for the purpose of extraction of phytochemicals present in the leaves. The extraction is carried out using different solvents such as n-hexane, ethyl acetate, methanol and aqueous in the order of increasing polarity. The extracts obtained in each solvent are collected separately and the solvents are evaporated using vacuum distillation and dried. The dried samples are stored in an airtight container for further analysis (7).

2.3 Phytochemical screening

The qualitative tests were carried out for different extracts of leaves of cappris zeylanica linn by adopting standard procedure (8-10). The crude extract were screened for the presence of phytochemicals.

1 Test for alkaloids

Small portion of solvent free extract was stirred with few

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drops of dil HCl and filtered. The filtrate was then tested for following colour test

Mayer's test:

(a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water.

(b) 5gms of potassium iodide was dissolved in 20 ml of distilled water (a) and (b) was mixed and the volume adjusted to 100ml with distilled water. Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

Phytochemicals	n-Hexane	Ethyl acetate	Methanol	Aqueous	
Alkaloids	+	+	+	+	
Amino acids	-	-	+	-	
Flavanoids	+	+	+	+	
Tannin	-	+	+	+	
Saponin	-	-	+	+	
Terpenoids	+	-	+	+	
Steroids	+	+	+	+	
Carbohydrate	+	+	+	+	
Cardiac glycosides	+	+	+	+	
Fixed for fat and oils	-	-	-	-	
Phenol	-	-	+	+	
Protein	-	+	-	+	
Reducing sugar	+	-	+	-	
Quinoline	-	+	-	-	

Table 3.1 Preliminary Qualitative Analysis of Cappers zeylanica linn leaves

(+) Present, (-) Absent

2 Test for flavonoids

Shinoda's test: 5 ml of 20% sodium hydroxide was added to equal volume of the extract. A yellow solution indicates the presence of flavonoids.

3 Test for steroids

Liebermann Buchard test: A small amount of sample is treated with 2ml of acetic an-hydride followed by the addition of 3ml of H2SO4 Solution. Color changes from violet to green or blue indicates the presence of steroids.

4 Test for terpenoids

Salkowski Test: To 1ml of extract add 0.5ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids.

5 Test for proteins and amino acids

Ninhydrin test: 1gm of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract was treated with this solution gave violet colour on boiling.

6 Saponins

Froth test: 5ml of extract is diluted with 20ml of distilled water and agitated for 10 minutes. Foam is formed which indicates the presence of saponins.

7 Test for Carbohydrates

Fehling test: Two milliliters of each plant extract were hydrolyzed with dilute HCl, neutralized with alkali, and then heated with Fehling's solution A and B. The formation

of a red precipitate was an indication for the presence of a reducing sugar.

8 Test for tannins and phenolic compounds

Lead Acetate test: 10% lead acetate solution, 0.5g of the extract was added and shaken to dissolved. A white precipitate observed

indicate the presence of tannins and phenolic compounds.

9 Test for fats and fixed oil

Spot test: Small quantity of the extract is placed between two filter papers. Oil stains produced with any extract shows the presence of fixed oils and fats in the extract.

10 Test for cardiac glycosides:

Keller-Killani test: To 2ml of extract, glacial acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Appearance of reddish brown color at the junction of the two liquid layers indicates the presence of cardiac glycosides.

11 Test for Quinones

Sulfuric acid test: One drop of concentrated sulfuric acid was added to 5 ml of each extract dissolved in isopropyl alcohol. Formation of red color indicates the presence of quinones.

2.4 Antimicrobial activity

The antimicrobial test is an essential technique used in pharmacology to investigate the efficacy and potency of antimicrobial agents from herbal extracts against microorganisms. (15). The pathogenic microorganism chosen for the antimicrobial activities are serratia.SP, Staphylococcus aureus, Bacillus Subtilis, Streptoccus mutans, Salmonella.SP, E-coli, Pseudomonus and Klebsiella.SP.

3 Results and Discussion

3.1 Qualitative analysis

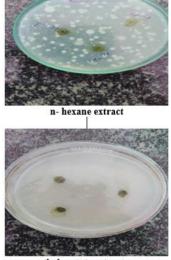
The crude extract of Cappers zeylanica linn leaves of different solvents was screened for the presence of phytochemicals and the result shows that the hexane extract of the plant leaves shows the presence of alkaloids, flavonoids, tannin, carbohydrate, glycosides, steroids, saponins, etc which is shown in the Table 3.1

3.2 Antimicrobial activity

The antimicrobial activity was performed by agar well diffusion method. The ethyl acetate and methanol extract from cappris zeylanica linn exhibited antimicrobial activity towards all the three micro organisms and the corresponding zone of inhibition values are given in the Table 3.2

Zone of inhibition in mm													
S.No.	Name of the mi-	n-hexane			Ethyl acetate		Methanol		Aqueous				
	cro organism	10-1	10-2	10-3	10-1	10-2	10-3	10-1	10-2	10-3	10-1	10-2	10-3
1	Bacillus subtilis	-	-	-	11	12	16	15	17	20	-	-	-
2	E- coli	-	-	-	12	12	13	14	17	19	-	-	-
3	Staphylococcus aureus	-	-	-	11	11	12	10	12	13	-	-	-

Table – 3.2 Antimicrobial activity of various extracts



ethyl acetate extract



methanol extract



aqueous extract

Figure 3.1 Anti microbial activity of n-hexane, methanol, ethyl acetate and aqueous extract

Bacillus subtilis was found to be maximum towards methanol extract with inhibitory zone of 20mm and minimum activity towards ethyl acetate observed with 11mm. E- coli was found to be maximum towards methanol extract with inhibitory zone of 19mm and minimum activity towards ethyl acetate observed with 12mm. Staphylococcus aureus was found to be maximum towards methanol extract with inhibitory zone of 13mm and also minimum activity towards methanol observed with 10mm. The n-hexane and aqueous extract of cappris zeylanica linn leaves does not show any antimicrobial activity towards all the three micro organisms.

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