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Characterization and In-Vitro Antibacterial Evaluation of Ethanolic Extract of Capparis Zeylanica Stem

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Abstract: Medicinal plants are becoming key sources for pharmaceutical preparations. The Indian Capparis zeylanica Linn belongs to the Capparidaceae family. The plant bitter root and bark are used as a tonic, expectorant, anthelmintic and painkiller, as well as for rheumatism, paralysis, toothaches, and enlarged spleen. The presence of different phytochemicals such as steroids, alkaloids, glycosides, and flavonoids was discovered during the phytochemical screening of this plant. So, in this work, we extracted and identified chemicals from the ethanolic extract fraction of Capparis. zeylanica stems, as well as evaluating the antibacterial activity of several Capparis zeylanica fractions. Sample S1 exhibited good action against gram +ve and gram –ve microorganisms at a concentration of 1 mg/ml, while Sample S3 showed moderate activity.

Key words: Characterization, In-Vitro, Ethanolic Extract, Capparis Zeylanica.

1. INTRODUCTION:

Long before the prehistoric period, plants were employed for medical purposes. Herbs were described in ancient Unani texts, Egyptian papyrus, and Chinese literature. Unani Hakims, Indian Vaids, European and Mediterranean cultures have all used plants as medicine for over 4000 years, according to evidence. [1]
Herbal remedies were employed systematically in indigenous societies such as Rome, Egypt, Iran, Africa, and America, while others produced traditional medical systems such as Unani, Ayurveda, and Chinese Medicine. Traditional medical systems are still frequently used for a variety of reasons. Increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments has resulted from population growth, insufficient drug supply, prohibitive treatment costs, side effects of several synthetic drugs, and the development of resistance to currently used drugs for infectious diseases. [2]
Treatment with medicinal plants is thought to be quite safe, with no or little adverse effects. The fact that these medicines are in tune with nature is the largest advantage. The golden truth is that herbal remedies may be used by people of all ages and genders. Medicinal plants are thought to be a rich source of components that can be utilized to make Pharmacopoeial, non-Pharmacopoeial, or synthetic medications. Apart from that, these plants have played an important role in the evolution of human cultures all across the world. Furthermore, some plants are regarded as vital sources of nourishment, and as a result, they are suggested for

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their medicinal properties [3-5]. Ginger, green tea, walnuts, aloe, pepper, and turmeric are just a few of these plants. Some plants and their derivatives are key sources of active compounds used in aspirin and toothpaste, among other things. Herbs are utilized in natural colouring, pest control, food, perfume, tea, and other applications in addition to medicine. Various medical plants/herbs are used in various nations to keep ants, flies, mice, and flee away from houses and businesses. Medicinal plants are becoming key sources for pharmaceutical production. [6-9]

The Indian Capparis zeylanica Linn belongs to the Capparidaceae family. It's a thorny, wiry shrub with a lot of branches that's found in Bangladesh, India, Sri Lanka, and Malaysia. Plants reach a height of 2–3 meters. Almost every component of the plant, including the root, bark, fruits, leaves, and seeds, is utilized for a variety of uses. The plant's bitter root and bark are used as a tonic, expectorant, anthelmintic, emmenagogue, and painkiller, as well as for rheumatism, paralysis, toothaches, and enlarged spleen. The root bark decoction is used as a deobstruent for the liver and spleen, as well as an anthelmintic and anti-inflammatory agent in Unani medicine. The presence of different phytochemicals such as steroids, alkaloids, glycosides, and flavonoids was discovered during the phytochemical screening of this plant. So, in this study, we attempted to isolate and characterize compounds from the ethanolic extract fraction of Capparis zeylanica stems, as well as to assess the antimicrobial activity of different fractions of Capparis zeylanica, and to compare the potency of isolated compounds to inhibit Gram-positive and Gram-negative bacteria. [10-13]

2. MATERIALS AND METHODS

Authentication of plant

Botanical Survey of India, Pune, Maharashtra, verified the plant specimen, which was collected in the Solapur area.

Preparation of the extracts

Capparis zeylanica stems were extracted by maceration with 80 percent alcohol and 20 percent water for 14 days. The extracted drug was then fractionated using the mother liquor process, which included hexane, ethanol, and ethyl acetate. Preliminary phytochemical screening was performed on these fractions, with several qualitative assays used to discover phytochemical elements present in all three fractions. On TLC over silica gel, the ethanol fraction revealed distinct dots. The ethanol extract was submitted to column chromatography since it contained more components than the other extracts. [14-16]

Column Chromatography of Ethanol fraction of Capparis zeylanica extract

Using silica gel as a stationary phase, normal Column Chromatography (CC) was employed to fractionate chemicals from fractions. Slurry was made by mixing 90g of silica gel for column chromatography (CC) with hexane and swirling it with a stirring rod. 25 gram of ethanol extract were combined with 1 gram of silica gel and dried at room temperature. To remove lipids, waxes, and some chlorophyll, hexane was progressively added to the column. The addition of ethanol enhanced the polarity (0 percent -100 percent). The entire amount of liquid utilized was 200 ml. A total of 50 fractions were collected and dried at room temperature. The fractions were blended based on the findings of the TLCs. The presence of steroids in fraction was discovered using concentrated sulfuric acid and vanillin as a spraying reagent. All of the compounds were crystallized, and three of them had their melting points

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recorded. To compute the Rf values, all of the compounds were exposed to TLC using a variety of solvent systems.

Identification of Phyto components
Whatman No.1 filter paper was used to filter the plant extract. IR, 1HNMR, and LCMS are some of the techniques used. After interpreting the spectra, IR, 1HNMR, and LCMS were obtained. The isolated compound's chemical structure is determined.

Antibacterial activity against different bacterial by well plated diffusion

Method:
The bacterial cultures were used to make the microorganism's inoculum. 15 milliliters of nutritional agar (Hi media) medium were placed into clean sterilized Petri plates and allowed to cool and harden. Standardized bacterial strain broth was pipette out and uniformly dispersed over the medium with a spreading rod until it dried. A sterile cork borer was used to drill 6mm diameter wells. All of the compounds were dissolved in DMSO at concentrations of 100, 200, and 300 g/ml. The wells were filled with 100 liters of plant extracts solution. The petri plates were incubated for 24 hours at 37°C. As a positive control, streptomycin (1 mg/ml) was made, whereas DMSO was used as a negative control. The diameters of the zone of inhibitions (ZI) were measured to determine antibacterial activity, and all measurements were done in triplicate. [17-19]

3. RESULT &DISCUSSION: -

Phytochemical Studies
Various qualitative assays were used to conduct preliminary phytochemical screening for Hexane, ethyl acetate, and alcoholic fractions of Capparis zeylanica. Table 1 shows the findings of the phytochemical screening. It is thought to be a molecule having steroidal nucleus based on the positive tests for steroids obtained by ethanolic extract (EEIC1). The Rf value of EEIC1 is 0.59, and it is a white crystalline material. The presence of steroidal nucleus is confirmed by the Rf value.

Table 1: Preliminary phytochemical analysis of fractions of Capparis zeylanica

Sr. No.	Test	Hexane fraction	Ethyl acetate extract	Ethanolic extract
i	Alkaloids	-ve	+ve	-ve
ii	flavonoids	-ve	+ve	-ve
iii	Carbohydrates	-ve	-ve	+ve
iv	Proteins	-ve	+ve	-ve
v	Tannins	-ve	-ve	-ve
vi	Steroids	-ve	-+ve	+ve

After IR, NMR, and LCMS analysis of the isolated substance EEIC1, it was shown to possess a steroidal nucleus, and its potential chemical structure is shown below (Figure 1).

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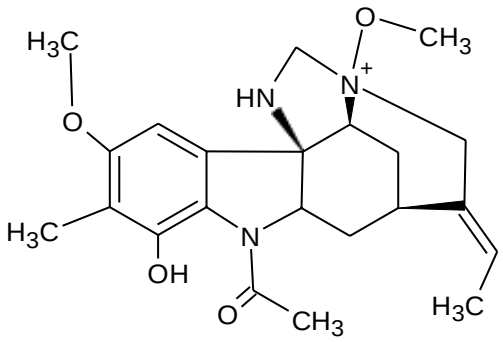

Figure1. Predicted Chemical structure of **EEIC1**

Molecular Formula: $C_{22}H_{30}N_3O_4^+$
Formula Weight: 400.4907514

Tableno.1 AntimicrobialActivity of5 extract against Shigella

Sr. No	SAMPLES	CONCENTRATION (mg/ml)	ZONEINDIAMETER (mm)
i	Control	-	0
ii	Standard (Streptomycin)	1	20
iii	S1	1	17
iv	S2	1	-
v	S3	1	9

Sample S1 exhibited excellent efficacy against Shigella at a dose of 1 mg/ml, whereas S3 showed moderate activity.

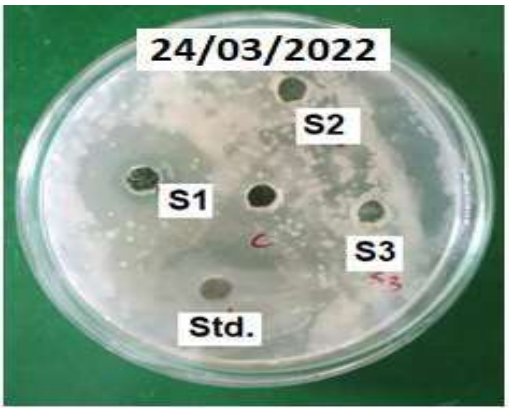

Figure 2. Activity of extract 1,2,3 against shigella

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Table2. AntimicrobialActivity of5 extractsagainst E.coli

Sr. No	SAMPLES	CONCENTRATION (mg/ml)	ZONE IN DIAMETER (mm)
i	Control	-	0
ii	Standard (Streptomycin)	1	19
iii	S1	1	13
iv	S2	1	-
v	S3	1	-

The Sample S1 had modest activity against E. coli at a concentration of 1 mg/ml.

Table3.AntimicrobialActivityof5 extracts against Bacillussubtilis

Sr.No	**SAMPLES**	**CONCENTRATION (mg/ml)**	**ZONEINDIAMETER (mm)**
i	Control	-	0
ii	Standard(Streptomycin)	1	29
iii	S1	1	16
iv	S2	1	-
v	S3	1	-

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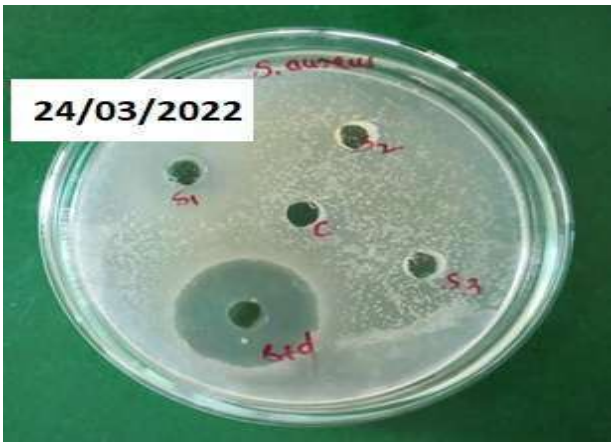
Sample S1 had modest activity against Bacillus subtilis at the various doses.

Tableno.4AntimicrobialActivityof4 extracts againstStaphylococcusaureus

Sr.No	SAMPLES	CONCENTRATION (mg/ml)	ZONEINDIAMETER (mm)
i	Control	-	0
ii	Standard(Streptomycin)	1	22
iii	S1	1	14
iv	S2	1	-
v	S3	1	-

Sample S1, S4 had modest activity against Staphylococcus aureus at the various doses.



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4. CONCLUSION

The antibacterial activity of Capparis zeylanica stem extracts was tested against five human pathogenic microorganisms. The inhibition zone on the plate showed that, the findings good and moderate effectiveness against all harmful bacteria tested.

Conflict of Interest
None declared by the author.

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