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In vitro antimicrobial activity of methanolic leaf extract of Cynara *scolymus* (artichoke) against selected human pathogens.

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Abstract

Medicinal plants are a reservoir of potentially valuable antimicrobial compounds that can be used to produce drugs as an alternative to synthetic microbicides. Methanolic leaf extract from Cynara scolymus was evaluated for its antimicrobial activity against 5 microbial pathogens, including two gram-positive: S. aureus, and E. faecalis, two gram-negative: P. aeruginosa and E. coli, and one yeast: C. albicans, using well diffusion assay technique. The leaf extract was found to be effective against all of the tested pathogens. The minimum inhibitory concentration (MICs) of the extract was determined by the broth dilution method. MIC values calculated were 64, 128, 64,128 and 128 µg/mL, which corresponds to E. faecalis, S. aureus, E. coli and P. aeruginosa, C.albicans respectively. Overall, Cynara scolymus methanolic leaf extract could be considered a promising source of antimicrobial agents.

Keywords**: **Medicinal plant, Cynara scolymus, antimicrobial activity, Agar well well-diffusion method, minimum inhibitory concentration

Introduction

Medicinal plants are a reliable source of naturally active compounds that can be used to protect human health and cure several diseases [1]. Alkaloids, phenols, tannins, flavonoids, vitamins, and minerals are examples of bioactive substances that include antioxidant, antitumor, antibacterial, anticarcinogenic, and diuretic properties [2]. As a result, research into the ethnomedicinal use and significance of herbal medicinal plants in the discovery of novel drugs is needed [3]. Plant derivatives are the source of the majority of synthetic medicines used to treat human illnesses [4]. The high incidence of multidrug-resistant bacteria, which is still waiting for successful antimicrobial drugs to be discovered, is an urgent demand on the pharmaceutical industry [5]. Plants must also be evaluated as a source of possible chemotherapeutic and antimicrobial agents, as well as their ethnomedicinal applications [6]. Natural antimicrobial agents, such as plant extracts of herbs or spices, are increasingly being

used to mitigate illness caused by pathogenic microorganisms [7]. *Cynara scolymus* is a native plant of the Mediterranean region that belongs to the family Asteraceae and genus *Cynara* [8]. Nowadays it is grown all over the world because of its nutritious and therapeutic qualities [9]. It has been used as a drug in traditional medicine since ancient times. It has been used in the treatment of biliary tract diseases/infections, digestive problems, and aids in the treatment of scurvy and anemia [10, 11]. It is not only a nutritious food with a pleasant bitter flavour but also a fascinating and widely used herbal medicine. Artichoke leaf extracts are often used alone or in combination with other herbs to bitter alcoholic and soft beverages, as well as to make herbal teas and medicinal items [12]. Artichoke intake is linked to antioxidant, hepatoprotective, hypoglycaemic, cardioprotective, anti-inflammatory, diuretic, antimicrobial, anticarcinogenic, and cholesterol-lowering properties [13]. The active constituents of this plant have been identified as polyphenolic compounds, found mostly in the leaves rather than the artichoke heads [14, 15]. The main aim of the study was to evaluate the antimicrobial activity of methanolic leaf extract of *Cynara Scolymus* against different human pathogens.

Materials and Methods

The Artichoke leaves were collected from higher reaches of the Daksum kokernag area of Anantnag Jammu & Kashmir, India, in July 2019. The plant sample was identified and authenticated by Centre for Plant Taxonomy (CBT), University of Kashmir. A reference specimen has been deposited in the KASH-Herbarium under voucher no.2835-KASH Herbarium

Fig1: *Cynara scolymus*

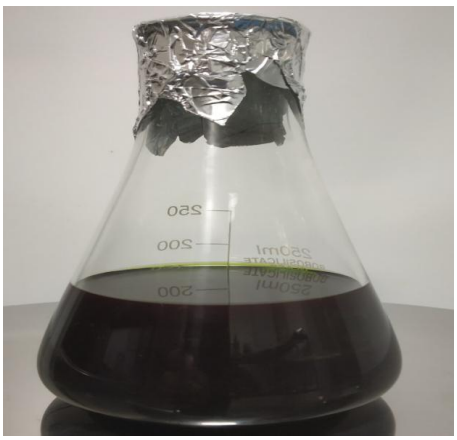


Fig 2: Methanolic extract of *Cynara scolymus*

The plant material (leaves) was shade dried at a temperature of 30 ± 3^0C. The dried leaf material was subjected to extraction by maceration using methanol as a solvent. The extract was then concentrated under room temperature and was stored in the refrigerator until further use.

Bacterial strains

The antimicrobial potential of plant extract was evaluated against five pathogens. Two gram negative stains *P. aeruginosa* ATCC 15442, *E. coli* ATCC 11229, and two gram positive strains *S. Aureus* ATCC 25923, *E. faecalis* ATCC 29212 and one fungus *C. albicans* ATCC 10231.

Antibacterial assays

The antimicrobial activity of the methanolic leaf extract of Cynara was determined using a well diffusion assay. For this, Muller Hinton Agar (MHA) medium was prepared and poured into sterile Petri plates. After solidification, the test pathogens at a concentration ranging from 10^4 to 10^6 CFU/mL were uniformly spread with sterile cotton swabs. The wells of 6 mm diameter were made on the surface of the medium, by a cork borer. Following that, different concentrations of the leaf extract 25 µl, 50 µl, 75 µl, and 100 µl from 10 mg per ml stock solution were introduced to the newly formed wells. Ampilox, ciprofloxacin, and fluconazole were used as positive controls for gram-positive, gram-negative, and *Candida albicans*, respectively. DMSO was used as a negative control. After 24 hours of incubation at 37°C, zones of inhibition were measured in mm. The experiment was done in triplicates for each tested pathogen and the data was expressed in mean±SD

Determination of the MIC

The experiment was carried out in 96 well plates flat bottom wells according to methods used by Gabrielson et al [16]. First ten wells containing 100 µL of serially diluted different concentrations of *Cynara scolymus* leaf extract (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL) were added 5 µL 12 hours old test pathogens. The plates were placed in an incubator at 37^0C for 24 hours. After incubation, the 10 µL of 5 mg/mL freshly prepared MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to all the wells and incubated for 2 hours. After that 100 µL of DMSO (Dimethyl Sulfoxide) solution was added as the solubilizing agent and colour change was observed visually. The colour change from yellow to purple was considered as positive. The minimum inhibitory concentration (MIC) of an extract was described as the lowest concentration that fully inhibited bacterial growth. The experiment was repeated three times.

Data Analysis

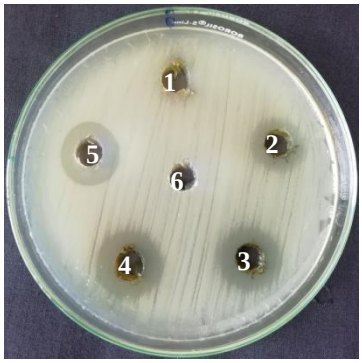
All of the experiments were carried out in triplicates separately, and the average zone of inhibition was measured using Microsoft Excel 2007.

Results and discussion

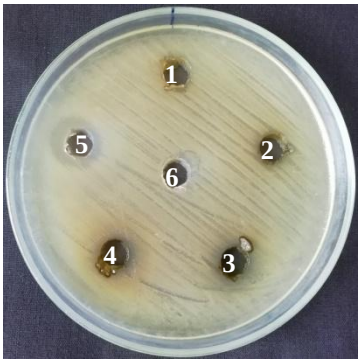
The growing number of multidrug-resistant pathogenic microorganisms in humans and animals, as well as the negative side effects of some antibiotics, has ignited huge interest in developing new antimicrobial drugs of plant origin [17]. In this study different concentration (25, 50, 75, and 100 µL) from 10 mg/ml stock solution of choke extract was screened for its antimicrobial activity against the selected pathogens. Table 1 and 2 shows the antimicrobial efficacy of choke extract against a group of five pathogens. The well diffusion assay revealed that methanol extract was effective against all test microorganisms, with the results being more pronounced against *E. faecalis* and *E. coli*, with the highest zone of inhibition 20 and 19 mm respectively, and the lowest MIC value of 64 µg /ml. The studies done by Pereira et al., 2016; Zhu et al., 2004 concluded that the antimicrobial properties of the artichoke were mostly attributed to the presence of phenolic compounds [18, 19]. The known antibacterial mechanisms of medicinal plants or their bioactive compounds against microorganisms include

inhibiting cell wall synthesis [20, 21] accumulating in bacterial membranes and causing energy depletion [22] or interference with cell membrane permeability, resulting in increased permeability, membrane disruption, loss of cellular constituents, changes in structure and finally cell death [23].

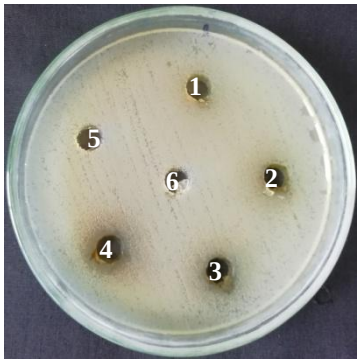
Fig.3. Antibacterial activity



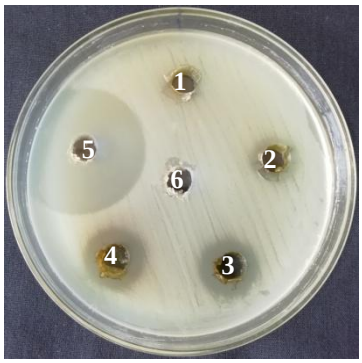
E. faecalis



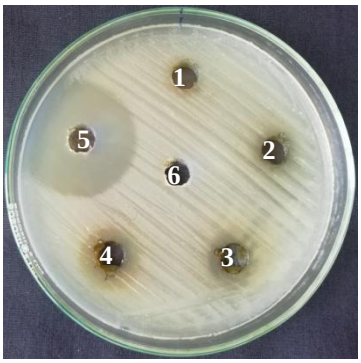
S. aureus



C. albicans



E.coli



P. aureginosa

1: 25 µl; 2: 50 µl; 3: 75 µl; 4: 100 µl; 5: SD: Standard drug: 10 µl 6: Negative control

Ampilox for Gram-positive: *E. faecalis*, *S. aureus*

Ciprofloxacin for Gram-negative: *P. aeruginosa*, *E. coli*

Fluconazole for fungi: *C. Albicans*

Negative control: DMSO

Table: 1Antibacterial activity of methanol extract of *Cynara scolymus* against human pathogens using well

S. No	Pathogen Name	Zone of inhibition in mm					
		25 µl	**50 µl**	**75 µl**	**100 µl**	**Standard drug 10 µl**	**Negative control**
1	*E. faecalis*	-	16	18	20	18	-
2	*S. aureus*	-	-	11	13	16	-
3	*C. albicans*	-	-	-	13	-	-
4	*E. coli*	-	12	17	19	35	-
5	*P. aeruginosa*	-	-	16	18	32	-

(-) denotes no activity, Inhibition zone: average of three independent experiments

Table 2. Minimum inhibitory concentration of the extract against human pathogens

Minimum inhibitory concentration (MIC) (µg/ml)				
Gram-positive bacterium		Gram-negative bacterium		Fungal pathogen
S. aureus	*E. fecalies*	*E. coli*	*P. aeruginosa*	*C. albicans*
128	64	64	128	128

Conclusion

In conclusion, we found that the methanolic leaf extract of *Cynara scolymus* had significant antimicrobial activity against all the tested pathogens and could be a promising source for the discovery of novel antimicrobial agents of plant origin.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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