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Evaluation of crude extract of *Catha edulis* for antimicrobial activity

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ABSTRACT

The emergence of new infections and increase of multidrug-resistance rises up the urgent need for the development of new antimicrobial agents from natural sources. This study was designed to prepare crude extract from leaves *Catha edulis* and evaluation for antimicrobial test. The micro-dilution method was applied for the determination of the minimal inhibitory concentration of selected reference pathogens. The antimicrobial activity of crude extracts was potentially inhibit the growth of Gram-negative bacteria comparing with Gram-positive bacteria. Crude leaf extract of *Catha edulis* showed the highest antibacterial activity against antibiotic resistant *Pseudomonas aeruginosa*; with a minimum inhibitory concentration value (200 mg/ml) lower than the reference antibiotic tetracycline (250 mg/ml). Higher inhibitory values (300 mg/ml to 500 mg/ml) were also obtained for crude extracts. Phytochemical analysis of crude extract of *Catha edulis* leaves presented the highest concentrations of amines, ascorbic acid, tannin, and alkaloids. These compounds have important biological activities and could be responsible for at least part of the antibacterial activity of the crude extract of leaves. The *Catha edulis* crude extract was the most efficient to inhibit the growth of the bacterial strain antibiotics resistant Pseudomonas aeruginosa, and Staphylococcus aureus, which represents an important step for the search and development of a new antimicrobial agent.

Introduction

Infectious diseases results a greater deaths approximately 17 million worldwide annually. Mostly in children and the elderly (Butler and Buss, 2006) the morbidity and mortality associated with infectious diseases have remained significant, particularly food-borne illnesses including diarrhea among children and respiratory infections such as tuberculosis, despite the advances in antimicrobial chemotherapy and supportive care. To make matters worse, the haphazard use of antimicrobials in the treatment of many infectious diseases has inevitably led to the emergence of multiple drug resistant microorganisms (Pandey and Madhuri, 2010).

For example, in 1990, almost all cholera isolates in New Delhi (India) were sensitive to furazolidone, penicillin, cotrimodal and nalidixic acid. In 2000, these drugs became largely obsolete in the treatment of cholera. The use of natural products,

such as medicinal plants as therapy against infectious diseases is a traditional therapeutic measure especially in developing countries as they contain a combination of potential antimicrobial compounds instead of a single purified molecule (Mishra and Mishra, 2011).

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Prakash and Gupta, 2005).

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Khan, 2010). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever bronchitis (Balakumar, et al., 2011; Joseph, 2013).

Even though microbial infections still now cause great troubles humanity due to lack of vaccine against some infections, especially pathogenic bacteria, emergence of drug resistance and the resurgence of infection among the others (Barron and Leung, 2015). In view of this, scientific studies have to be conducted on the traditional medicinal plants to overcome the global problem of antimicrobial resistance and for the purpose of developing a new, effective and safe antimicrobial drug. It was necessary to further study its antibacterial activity by using crude extract, which in turn could simplify the isolation and identification of active principle responsible for the antimicrobial activity of *Catha edulis*.

Materials and methods

Chemicals and materials

For this study methanol (solvent), distilled water dimethyl sulfide, Muller Hinton agar, nutrient agar, peptone water, penicillin, crystal violet iodine, alcohol, cotton swab and safranin were used.

Test organisms

Human pathogenic microorganisms namely, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* were used for the antimicrobial activity test.

Collection of *Catha edulis* and sample processing

The leaves of *Catha edulis* (Fig. 1) were collected from farm, in Ejersa Fora kebele (Hule Hora town) in West Guji zone of Oromia. The fresh samples were packed in plastic bags and transported into the laboratory in the month of March 2019. The leaves of *Catha edulis* were collected and washed with distill water to remove dirt and other contaminants. Then they were washed with 10% saline solution and again with distilled water. After that, the leaves were shade dried until the moisture is reduced to 10%. The dried leaves were blended into powder form.

Fig. 1: Fresh *Catha edulis* leaves.

Crude extract preparation

The crude extract was prepared from the leaves of *Catha edulis* by solvent extraction (Harborne, 1998) and Soxhlet extraction (Kokate et al., 1995). In solvent extraction, the dried plant sample were taken and required solvent in the ratio (1 g: 20 ml) respectively in an amber colored glass container with air tight lids for 4 days with frequent agitation. Then they were filtered and the filtrate is the crude extract. In Soxhlet extraction, the sample was loaded into the thimble and solvent in the bottom flask/boiling flask. The cycles were continued until all the extract has been exhausted from the plant sample (generally 10 cycles). The flowchart for crude extract preparation is given in Fig. 2.

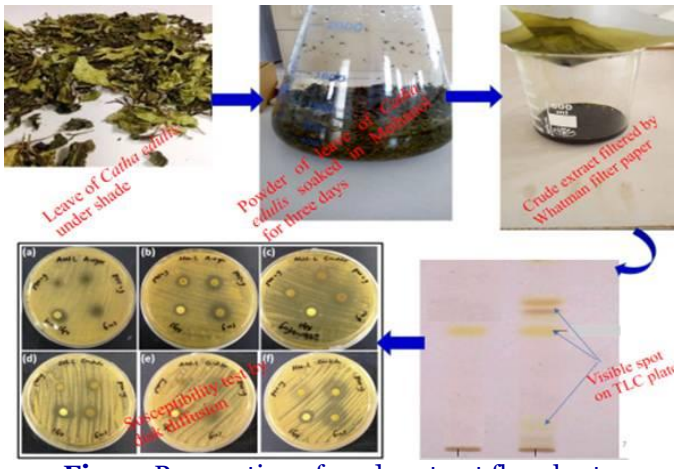

Fig. 2: Preparation of crude extract flowchart.

Qualitative screening of secondary metabolites

The presence of alkaloids, flavonoids, glycosides, carbohydrate, saponins, tannins and terpenoids can tested qualitatively using the standard procedures to identify the constituents (Gogoiand and Islam, 2012).

Thin layer chromatography

In thin-layer chromatography, the stationary phase is a polar absorbent, usually finely ground alumina or silica particles. This absorbent is coated on a glass slide or plastic sheet creating a thin layer of the particular stationary phase. Almost all mixtures of solvents can be used as the mobile phase. By manipulating the mobile phase, organic compounds can be separated (Karthikeyan et al., 2009).

Preparation of filter paper discs and Minimum Inhibitory Concentration

Filter paper is used to prepare discs approximately 6 mm in diameter, which are mix with crude extract of *Catha edulis* leaves and placed in a Petri dish and sterilized in a hot air oven at 160°C for 180 minutes. Both bacteria are grown in 10 ml of peptone water respectively for various concentrations. After 12 hours of incubation at 37°C, different concentrations of *Catha edulis* extracts ranged from 0, 50, 100, 200, 300, 400 and 500 mg were added in various tubes of grown bacteria and again incubated for 10-12 hours at 37°C. After incubation we observed the growth pattern in UV-spectrometer at 320 nm wavelength for all concentrations, and further analyzed for the minimum inhibitory concentration and the lethal dose of the *Catha edulis* against bacteria.

Antimicrobial activity assay

The antimicrobial activity test of the crude *Catha edulis* extracts against pathogenic organisms were carried out by Disk Diffusion method. Muller Hinton Agar and Potato Dextrose agar were prepared for each organism as follows. 0.2 ml of the standardized inoculation were mixed with 20 ml of sterile agar (maintained at 45-50°C in a molten state) using a mixer, and then poured into sterilized Petri dishes and set aside. After congealing, well-isolated colonies of the same morphological type are selected from an agar plate cultured at 37 °C for 18-24 hours. The predetermined lethal doses (350 mg and 340 mg/ml with disc) crude of *Catha edulis* are dispensed onto the surface of the incubated agar plate.

Each disc was pressed down to ensure complete contact with the agar surface and the discs are placed with a dispensing apparatus, they distributed evenly. Ordinarily, 4 discs were placed on each plate. Each the plates are inverted and placed in an incubator set at 37°C for 18 hours after the discs are applied. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition excluding the whole size by use of an antibiotic zone reader.

Data analysis

The data for this study were analyzed by using descriptive form of statistics and also it was represented in the form of tables and figures.

Results and discussion

Phytochemical screening

The plant samples were dried and the solvent system used was Methanol: Water (70:30), both for solvent extract and Soxhlet extract. Qualitative analysis was carried out for the solvent extract and Soxhlet extract and the results are shown in Table 1.

Table 1. Presence of secondary metabolites in *Catha edulis*.

Extracts	Alkaloids	Saponin	Tannin	Flavonoids	Terpenoids
Solvent extract	+	_	+	+	_
Soxhlet extract	_	+	+	_	_

NB: - absence, + presence

Antimicrobial activity test

The results of antimicrobial activity of *Catha edulis* leaf extract against *Staphylococcus aureus Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* with different concentrations revealed antimicrobial effect. The least zones of inhibition were displayed by the negative control and Gentamycin exhibited the widest zones of inhibition against all the bacteria. *Catha edulis* leaf extract showed increasing zones of inhibition. Results of the study as showed in the table *Catha edulis* have a potent antimicrobial activity. The activity of *Catha edulis* extract against *Klebsiella pneumoniae* and *Staphylococcus aureus* was found to be higher at a concentration of 100% followed by 75% and 50% respectively. The maximum zone of inhibition was found to be 21 and 11 mm against *Klebsiella pneumoniae* and 18 and 15 mm against *Staphylococcus aureus*.

Estimation of MIC and lethal dose

The results of spectrometer studies implied 350 mg/ml for *Staphylococcus aurous* and 340 mg/ml for *Pseudomonas aeruginosa* were the lethal dose crude extracts of *Catha edulis* extracts (Fig. 3).

Fig. 3 shows that as concentrations of crude extract of *Catha edulis* increase, the inhibition effect on the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* and *Candida albicans* also increase.

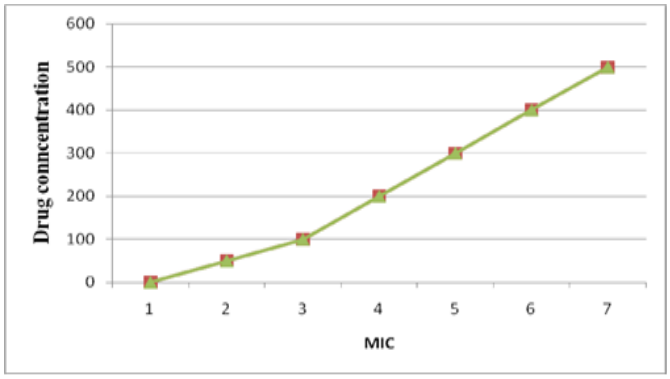

Fig. 3: Antimicrobials activity crude extract of *Catha edulis* against at various concentrations.

Zone of Inhibition

Antimicrobial activity crude extract from leaves of *Catha edulis* were evaluated for different concentration. Based on diameters of inhibition zone around filter paper disks, if there is no inhibition zone, it is assumed that there is no antimicrobial activity. Fig. 2 shows that representative disk diffusion plates with initially our selected bacteria after 24 hours incubation. The diameter of inhibition zone of *Pseudomonas aeruginosa* was larger than that of *Staphylococcus aureus*, indicating that *Pseudomonas aeruginosa* is more susceptible to crude extraction of leaves of *Catha edulis* than *Staphylococcus aureus*. Table 1 shows the antimicrobial activity of leaves of *Catha edulis* comparatively with against Gram positive bacterial strains such as *Staphylococcus aureus,* and Gram-negative bacterial strains such as *Pseudomonas aeruginosa* by disk diffusion. With regards to diameters of the inhibition zones, crude extraction leaves of *Catha edulis* demonstrated that effective inhibition on the growth of these bacteria. Among two bacteria, *Pseudomonas aeruginosa* was significantly more susceptible while *Staphylococcus aureus* was more resistant.

The average size of inhibition zones varied from 5mm to 18mm against *Pseudomonas aeruginosa*, and 2 to 8 mm against *Staphylococcus aureus* compared to standard drug inhibition by

tetracycline 6 mm to 25 mm showed significant results as compare to tetracycline. However, as the concentration of crude extraction leaves of *Catha edulis* increase, inhibition effect on the growth of *Pseudomonas aeruginosa*, *Staphylococcus aurous Escherichia coli Klebsiella pneumonia* and *Candida albicans* were increased. This shows that higher antimicrobial activity at higher concentration had clear inhibition zones. The, inhibition zone sizes increased at a higher crude extractions leaves of *Catha edulis* concentration. Most of test organisms, which indicated leaves of *Catha edulis* was more effective at higher concentration against antibacterial.

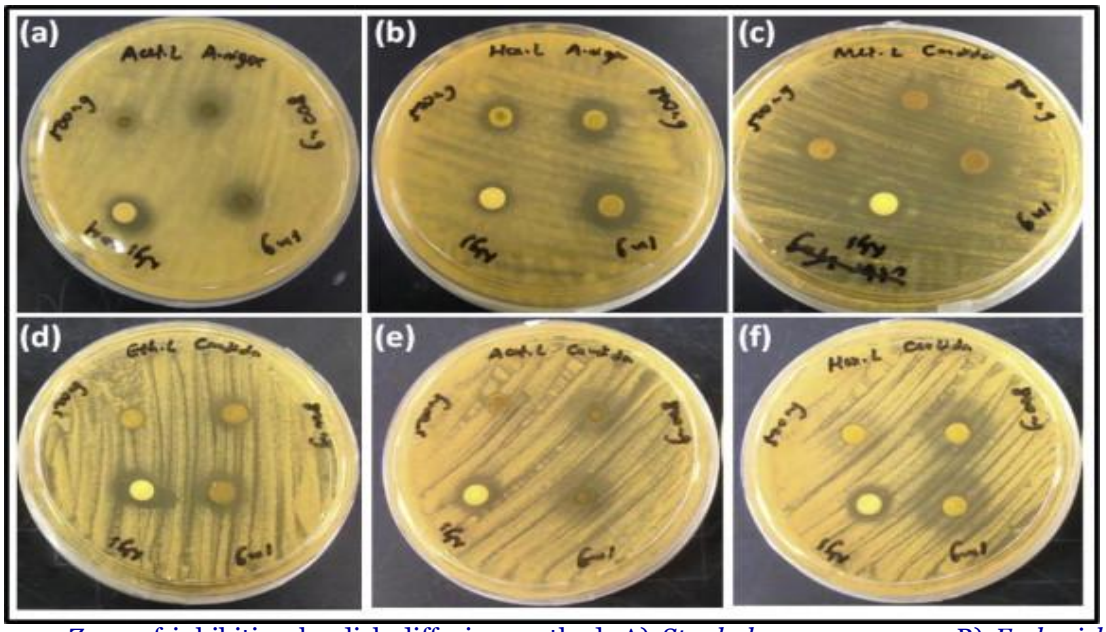

Fig. 3: Zone of inhibition by disk diffusion method. A) *Staphylococcus aureus,* B) *Escherichia coli,* C) *Pseudomonas aeruginosa*, D) *Klebsiella pneumoniae,* E and F) *Candida albicans.*

Table 1. Minimum inhibitory concentration (MIC, in 350 and 340 mg/ml) of crude extractions obtained from leaves of *Catha edulis* on test organisms at different concentration.

Test organisms	**Zone of inhibition**	
	Gentamycin	**Crude extract of** *Catha edulis*
Staphylococcus aureus	24 cm	13 cm
Escherichia coli	22 cm	9 cm
Pseudomonas aeruginosa	26 cm	17 cm
Klebsiella pneumoniae	18 cm	5 cm
Candida albicans	.....	10 cm

Conclusion

The findings of the present study were demonstrated that the potential of crude extraction leaves of *Catha edulis* against antimicrobial activity is a natural source, in the pathway of developing an antimicrobial agent able of treating bacterial infections. Amines, Ascorbic acid, tannin, and alkaloid Chemical that found in leaves of *Catha edulis* showed promising results against a penicillin resistant *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella* which represents an important step for the search and development of a new antibacterial agent. Further toxicological and pharmacological studies will be useful to confirm of crude extract leaves of *Catha edulis* against antibacterial activity by the same procedure or in other methods.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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