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Antagonistic effects of *Ageratum conyzoides* (crude extract) against *Escherichia coli*

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Abstract
Ageratum conyzoides has a wide range of pharmacological activities. *E. coli* strains and its virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In this work different concentration shows as 100, 75, 50, 25, and under controlled gave inhibition zone as 7.33, 6.00, 5.33, 2.66, but controlled patriplate full of microorganism.

Keywords: triclosan, TCS, determination, detection, sensor

Introduction
Medicinal plants have played an essential role in the development of human culture, for example religious and different ceremonies. (E.g. Datura has long been associated with the worship of Shiva, the Indian God). Durba grass (*Cynodon dactylon*) is believed to have a purifying effect on participants and is used as an offering in Ganesha Temples.
Ever since ancient times, in search for rescue for their diseases, the people look forward for drugs in nature. The beginnings of the medicinal plant's use were instinctive, as in case with animal. In view of the fact that at that time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, and everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of chemistry (a school of thought of the 16th and 17th centuries which sought to understand combination of medicine and physiology in terms of chemistry) in 16th century, plants had been the source of treatment and prophylaxis. Nonetheless, the efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again. Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and with the advancement of civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. The benefits of one society were passed on to another, which upgraded the old properties and discovered new ones, till present days.
The use of plant compounds to treat infection is an age old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases. According to World Health Organization medicinal plants would be the best source of Ayurveda In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor *et.al.*, 1995, Ratnakarand Murthy; 1995; Silva *et.al.*, 1996, David; 1997, Saxena; 1997, Nimri *et.al.*, 1999, Saxena and Sharma; 1999) [4, 5, 8, 9, 1014, 15]. The selection of medicinal plants is based on their traditional uses (32 plants) in India and our reported antimicrobial activity of 13 plants (Chopra *et.al.*, 1992) [3]. However, most of these plants were not previously screened against multi-drug resistant, pathogenic organisms.

Microorganisms
Microorganisms (or microbes for short) play a very important role in our lives. Some microbes cause disease but the majority of them are completely harmless.

In fact we couldn't live without them, but they could live without us. These microscopic organisms play a key role in maintaining life on earth, fixing gases and breaking down dead plant and animal matter into simpler substances that are used at the beginning of the food chain. Biotechnologists can also exploit the activities of microbes to benefit humans, such as in the production of medicines, enzymes and food. They are also used to breakdown sewage and other toxic wastes into safe matter. This process is called bio-remediation.

Microbes are very small living organisms, so small that most of them are invisible. The majority can only be seen with the help of microscope, which magnifies their image so we can see them. In fact microbes are so tiny you would find over a million in a teaspoon of soil. They make up more than 60% of the Earth's living matter and scientists estimate that 2-3 billion species share the planet with us. Plants are sources of very potent and powerful drugs with antibacterial properties (Chopra *et.al.* 1992 [3]. They can be used in the treatment of infectious diseases caused by resistant microbes (Bhattarai and Shreshta; 2009) [2].

Material and Method

1. Plants of *Ageratum conyzoides* was collected from Dehradun valley. The plant was identified by Botanical Survey of India, Dehradun. The roots of *A. conyzoides* were chopped off leaving the aerial part which was shredded into tiny bits. Traditional communities in India use this species as an anti-dysenteric and antilithic aqueous extract of this plant is used as an antibacterial agent, Singh BR (2013) [16], Bhattarai N. and Shrestha G. (2009) [2].

Ethno pharmacology

A. conyzoides has a wide range of pharmacological activities. The pharmacological effects reported highlightened anti ulcerogenic, anti-allergic, anti-inflammatory, anti-cataleptic, anti-diabetic, anti-tumour, radio protective, anti-todal, antioxidant, anti-protozoal, anti-microbial, anthelmintic, allelopathic, insecticidal, hematopoietic, wound healing, gastroprotective, uterine and bronchodilating potential of *A. conyzoide* Saxena, K (1997) [14]. Apart from these uses, the ethnoveterinary use of the plant in the management of diarrhoea and coccidiosis in livestock has also been scientifically confirmed. The plant can also be used as natural herbicides to control weeds to reduce the consumption of synthetic herbicides. Precocene 1, Precocene 2 and coumarin compounds are the major constituents of the plant which have been reported to be biological active compounds Precocene1, precocene2 has been used as insect regulators by inducing symptom of juvenile deficiency hormones in insects. Various extracts of the plant, including water and methanol have been shown to inhibit the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and H. pylori* (Vadhana P, et. al. (2015) [17]. Iyengar, M.A. (1985) [7].

Escherichia coli

Escherichia coli also known as *E. coli* is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

Role of *E. coli* in causing disease

Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome, however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli*.

Method

The plant material was washed gently with Tin twenty to remove any dirt and to free it from any microbe and was air-dried under shade for a week. The sample was powdered with an electric grinder into a coarse form and stored in airtight containers. Akinyemi, *et.al*. (2005) [1].

Plant Material	Fresh weight	Dried weight
Ageratum conyzoides (L).	500 grams	150 grams

After grinding, 30 gm of plant material was extracted in 130 ml of methanol for 24 hours. The extracts were filtered through Whats-man filter paper and were evaporated to dryness using a hot plate at temperature (40℃). The residues obtained were dissolved in methanol. The weights of the extract was determined and stored below ambient temperature. Vandana *et. al*., 2015, Singh 2013, Okwari *et. al*. 2006 [13, 16, 17].

Culture collection and maintenance

Strains of *Escherichia coli* were used for the study of antibacterial activity. These standard strains were prepared in microbiology laboratory of UCBMSH, Dehradun.

1. Reagents

1. Muller Hinton Agar Medium (250 ml)

The medium was prepared by dissolving 19.5 g of the commercially available Muller Hinton Agar Medium (Hi Media) in 250ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 10 plates petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (50 ml))

50 ml of nutrient broth was prepared by dissolving 0.25g beef extract, 0.25g Peptone, 0.15g Nacl in 50 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Micro dilution Method

Procedure

The procedure follow serial dilution principle with the result of minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted plant extracts. The methanol extract were diluted to get series of concentrations from 25%, 50%, 75% and 100% respectively in distilled sterilized water. The microorganism suspension of 50µl was added to the broth dilutions. These were incubated for 18 hours at 37ºC. MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth containing crude Methanolic extract of *A. conyzoides* with different concentrations.

Evaluation of antibacterial activity

The effect of various plant extracts on the several bacterial strains were assayed by Agar well diffusion method and further confirmed by Disc diffusion method. The minimum concentrations of the plant extracts to inhibit the microorganisms were also determined by micro dilution method using plant fractions serially diluted in sterile nutrient broth. Bacterial cultures of that were used for antimicrobial assay of test organisms were obtained from the culture collection. The bacteria were maintained on nutrient broth (NB) at 37℃ till required for analysis.

Agar well diffusion method

Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Result and Discussion

Results of the antibacterial screening of different concentrations of the extracts on the test isolates are tabulated in Table 1. The results show that the increase in concentration of the extract increased the zones of growth inhibition of the bacteria. The assessment of the antibacterial activity was based on the measurement of diameter zone of inhibition (mm) that formed around the hole made by the borer filled with the extract. Maximum inhibition zone was recorded at 100 mg/ml and the minimum inhibition zone at 25 mg/ml in both the bacteria for all the extracts and controlled petriplates showed no zone of Inhibition. This proved that the extract is effective against *E.coli* microbes.

In this study, the results obtained indicated that the methanolic extract of the plants inhibited the growth of the two test bacteria. This therefore, showed that the extract contained substances that can inhibit the growth of the selected bacteria. Other workers have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations. *A. conyzoides* a wide range of chemical compounds ISncluding alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated. Phytochemical screening of extracts of *Ageratum conyzoides* showed the presence of alkaloids, saponins, flavonoids, tannin, steroids and cardiacglycosides so, it may be due to the presence of these chemical compounds and substances that the plant extracts can inhibit the growth of the bacteria.

Table 1: Inhibition effects of *A. conyzoides* extract on *Escherichia coli*

Concentrations of *A. conyzoides* extract (mg/ml)	Zone of Inhibition (mm) *Escherichia coli*
100	7.33
75	6.0
50	5.33
25	2.66
Controlled	0.0

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