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Antibacterial activity of *Arctostaphylos uva-ursi* leaf extract against urinary tract infection causing agents

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Abstract
Urinary tract infection is one of the most prevalent illnesses seen in modern medicine, affecting patients of all ages from newborns to the elderly. Superficial urinary tract infections can still be easily treated with a brief course of antibiotics, although resistance to many of the antibiotics used to treat these infections is growing. Plants produce a wide range of bioactive chemicals, making them an abundant source of several types of medications. The majority of medications used in traditional medical systems today are either from natural sources or semi-synthetic derivatives of natural items. Therefore, screening conventional natural compounds makes sense in the drug discovery process. This study has been focused to determine the antibacterial activity of *Arctostaphylos uva-ursi* leaf extract against urinary tract infection causing agents. The extract showed excellent antibacterial activity against the tested microorganisms which can gain attention of pharmaceutical industry for preparation of antimicrobial agents of natural origin.

Keywords: UTI, antibacterial activity, *Arctostaphylos uva-ursi*, leaf extract

1. Introduction
A bacterial illness that affects a portion of the urinary tract is known as a urinary tract infection (UTI). It is referred to as simple cystitis (a bladder infection) when it affects the lower urinary tract and pyelonephritis (a kidney infection) when it affects the upper urinary tract. Lower urinary tract symptoms include painful urination, frequent urination, or the need to urinate, or both; on the other hand, symptoms of pyelonephritis include fever and flank discomfort in addition to lower urinary tract symptoms. The extremely young and the elderly may have nonspecific or ambiguous symptoms. *Escherichia coli* is the primary cause of both types; however, in rare cases, other bacteria, viruses, or fungus may also be the cause. A complex UTI refers to infections in the urinary tract that have anatomical or functional abnormalities or the presence of foreign items, such as an indwelling urethral catheter. This approach, however, does not always represent clinical management. In children, a simpler and more practical strategy is to differentiate UTI as a first infection from recurrent infection. Recurrent infections can be further classified into three types (1) unresolved bacteriuria, (2) bacterial persistence, and (3) reinfection (Chang *et al*., 2020) [1]. UTI is one of the most prevalent prevalent illnesses in modern medicine affecting patients of all ages, from newborns to the elderly (Kunin, 1994; Raju and Tiwari, 2004) [2, 3].
Women are more likely than males to get urinary tract infections, with half of them experiencing at least one infection throughout their lifetime. Female anatomy, sexual activity, and family history are risk factors (Schaeffer *et al*., 2001) [4]. Pyelonephritis is primarily caused by a bladder infection, but it can also be caused by a blood infection. Young, healthy women can be diagnosed only on the basis of their symptoms. Diagnosing people with hazy symptoms can be challenging since bacteria can exist without an illness. Urine cultures can be helpful in complex illnesses or in circumstances when treatment has not worked. As a preventative precaution, low dose antibiotics may be used to individuals who get infections frequently.
Superficial UTI can still be easily treated with a brief course of antibiotics, although resistance to many of the antibiotics used to treat these infections is growing. If symptoms have not improved after two or three days, additional diagnostic testing is required. In more complex cases, a longer course of treatment or intravenous antibiotics may be required. Ten percent of women experience urinary tract infections each year, making them the most common type of bacterial infection.

Plants are a rich source of various medicines because they produce a wide variety of bioactive molecules. The majority of medications used in conventional medical systems today come from natural sources or are semi-synthetic derivatives of natural products. In order to discover new drugs, screening conventional natural products makes sense. A significant number of new antibiotics have been introduced, and about 20% of all plants have been the subject of biological or pharmaceutical testing. According to reports, from 1983 to 1994 (Cragg *et al*., 1999) [5], systematic screening of antibacterial plant extracts was conducted in an ongoing effort to identify novel compounds that could potentially combat multi-resistant bacteria. Numerous researchers from all over the world have studied the antimicrobial properties of a few medicinal plants. The World Health Organization (Santos *et al*., 1995) [6] states that the greatest place to find a wide range of medications is from medicinal plants. One such medicinal plant with effective antimicrobial property against the agents of UTI is *Arctostaphylos uva-ursi*.

The Blackfeet Nation historically used bearberry leaves and fruits as food. The fruits can be used to make jelly, but even though they are edible raw, they are rather bland that way. The berries were cooked with meat and used as a seasoning. Tea can also be prepared from the young leaves. For centuries, First Nations people have used teas and extracts of the leaves as laxatives, diuretics, and antiseptics for the urinary tract. In herbalism, urinary tract inflammation is treated with leaf tea. Despite being believed to be an astringent or a treatment for STDs, there was, as of 2017, no solid proof from clinical studies demonstrating the efficacy or safety of such treatments. Known collectively as "kinnikinnick" (Algonquin for "smoking mixture"), dried bearberry leaves are the primary ingredient in a large number of traditional Native American smoking mixes that are used, particularly among western First Nations. Other herbs and occasionally tobacco are also added. The plant was also used by Native Americans to create yellow dye.

Many cultivars are produced specifically to be used as decorative plants. Its deep roots help to prevent erosion on hillsides and slopes, making it a year-round attractive evergreen groundcover for gardens. It is a common groundcover in urban areas, naturalized areas, and native plant or rock gardens because it can withstand sun exposure and dry soils.

2. Description of plant used in the study

Arctostaphylos uva-ursi is a small, woody groundcover shrub that grows to a height of 5 to 30 centimeters (2 to 12 inches). It is procumbent. The species can have dense wild stands that are rarely taller than 15 cm (6 in). Single roots form long, flexible prostrate stems from which erect branching twigs grow. Periodically, the trailing stems will send out small roots as they layer. When fully grown, the velvety branches with fine texture turn from pale green to white, eventually becoming smooth and reddish-brown in color. The tiny, lone, three-scaled buds have a dark brown color. The leaves are about 4 cm (1+1⁄2 in) long and 1 cm (1⁄2 in) wide, shiny, small, and feel thick and stiff. Their undersides are a lighter green color than their tops. Their rounded tips taper back to the base, and a twisted leaf stalk arranged alternately on the stem holds them vertically. After a period of one to three years, the leaves turn pale on the underside and turn reddish-green or purple before falling in the autumn. May and June see the terminal clusters of tiny urn-shaped flowers bloom. The drupes, which are round, fleshy or mealy, bright red to pink fruits, are produced by the white to pink flowers. The fruits have smooth, glossy skin and measure between 1/4 and 1/2 inch (6 and 13 mm) in diameter. The plant bears red fruits well into the early winter months. Raw fruits have a bittersweet taste, but after boiling and drying, they become sweeter. There are one to five hard seeds in each drupe, and in order to break embryo dormancy and reduce the seed coat before germination, the seeds must be scarified and stratified. Per pound, there are, on average, 40,900 cleaned seeds.

Fig 1: *Arctostaphylos uva ursi* plant a) Flowers b) Fruit

3. Materials and Methods

3.1 Collection of sample

The *Arctostaphylos uva ursi* leaf powder was procured from Amazon

3.2 Extraction of bioactive metabolites from *Arctostaphylos uva ursi* leaf powder

After mixing 10 g of the powder that had formed with 100 ml of distilled water, it was placed in a water bath heated to 60 ºC for half an hour. The extract was then filtered through Whatman filter paper no. 1 and kept for later use at 4 ºC.

3.3 Antibacterial activity

The agar disc diffusion method was used to test the antibacterial activity. The microorganisms utilized in the present investigation were procured from Microbial Type Culture Collection (MTCC) Chandigarh, Punjab.

3.4 Agar Disc Diffusion Method

- The plant extract's antibacterial activity was assessed using the Agar disc diffusion method, as per Bauer *et al*., 1966) [7].
- 20 ml of Muller Hinton Agar were added to sterile petri plates in order to assess the antibacterial activity using the agar disc diffusion method.
- The solidified media was covered with standard bacterial suspension inoculums adjusted to 0.5 McFarland turbidity standard, or 1 x 10^8 CFU/ml of bacteria. The inoculums were then swabbed on top and left to dry for ten minutes.
- 6 mm sterilized filter paper disks (Whatmann No. 1) were saturated with previously prepared plant extract at concentrations of 0.25 mg/ml.
- The impregnated discs were subsequently positioned on top of the agar medium that had solidified. After that, petri plates were incubated at 35±1^{0}C for 24 hours.
- After a 24-hour incubation period at 35±1^{0}C, the zone of inhibition created by the plant extract was finally measured. Distilled water was utilized as the negative control and the effects were contrasted with those of standard streptomycin (positive control).

- The study was conducted in triplicate, and the average values ± standard deviations were reported as the findings.

4. Results and Discussion

Plants contain a bizarre of metabolite which has marked antimicrobial activity, of course to survive in their natural environmental condition. Therefore it is not surprising that antimicrobial compound with more or less strong activities could be isolated from plants and that they could be of benefit for humans.

Medicinal plants are very rich in their phytochemical constituents which are prevalent and vary according to the species of the plants. This study was undertaken with the aim to evaluate antibacterial activity of *Arctostaphylos uva ursi* leaf extract against urinary tract infectious agent. As the reports available about the medicinal value of plant it was procured from Amazon.

Aqueous extract of the plant leaf was investigated for the activity. The inhibitory activity of crude aqueous extract was investigated against one gram positive and four gram negative bacteria which are medically important and responsible to cause UTI's. Preliminary antibacterial screening was performed in this investigation to determine whether the extract possessed any biological activity.

Table 1: Primary Screening of *Arctostaphylos uva ursi* for Potential Antibacterial Activity

S.N.	Name of plant	Common name	Family	*E. coli*
1.	*Arctostaphylos uva ursi*	Bear berry	Ericaceae	++

Table 2: Antibacterial activity of the plant extract against potential UTI causing bacteria. Mean±S.D, n = 3

S. No	Microorganism	Zone of inhibition (mm)		
		Plant Extract	Control	
			Positive	Negative
1.	*Salmonella enterica*	16.5±0.10	17	0
2.	*Staphylococcus aureus*	30.2±0.20	34.6±0.20	0
3.	*Klebsiella pneumoniae*	15.7±0.20	18.2±0.20	0
4.	*Escherichia coli*	20	22.5±0.30	0
5.	*Pseudomonas aeruginosa*	18.6±0.30	19.7±0.40	0

During the course of present investigation the potential antibacterial activity of aqueous extract of the test plant i.e. *Arctostaphylos uva ursi* was determined. It is evident from the data recorded in table 1 that the extract obtained from the test plant showed significant activity against one Gram negative bacteria. Antibacterial potential of the test plant was further determined against four gram negative (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica,* and *Escherichia coli*) and one gram positive bacteria (*Staphylococcus aureus*) pathogenic bacterial strains by using agar disc diffusion method. The plant extract was used at 0.25 mg/ml against all the microorganisms. The antibiotic streptomycin was used as a standard against bacteria and distilled water as negative control in this assay. It was found from the present study that the plant extract showed significant antibacterial activity against the test organisms, among which the gram-positive bacteria *Staphylococcus aureus* showed highest zone of inhibition, 30.2±0.20 mm. A study carried out by Dragana M *et al*., 2013 [8] reported the enhanced antibacterial activity of aqueous extract of leaf *Arctostaphylos uva ursi* support our results.

The antimicrobial activity of *uva ursi* is attributed to its power to change microbial cell surface characteristics. Presence of *uva ursi* extracts has the ability to increasing the microbial cell surface hydrophobicity, thus in turn decreasing their ability to cling to host cells. Antibacterial effects of the herb is exhibited by the existence of hydroquinone, originated from arbutin. It has antimicrobial and astringent properties when the stomach absorbs it and changes it into a substance with disinfectant. Arbutin fights with infection, soothes irritation and reduce inflammation during urination (Geetha R.V. *et al*., 2010) [9].

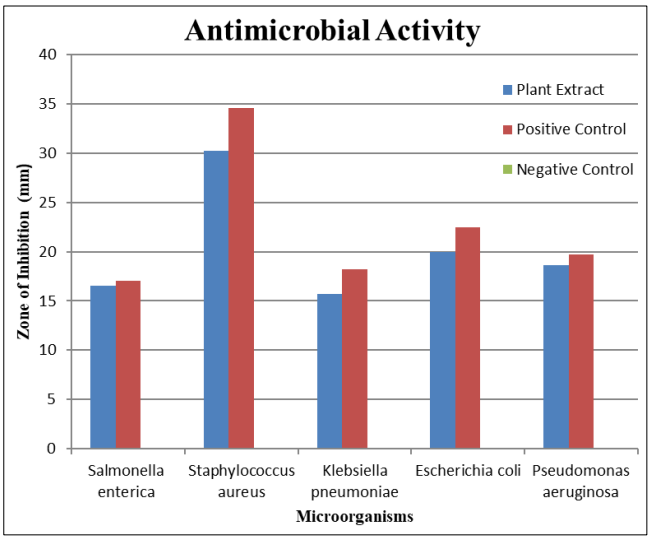

Fig 2: Graphical representation of antimicrobial activity of aqueous leaf extract of *Arctostaphylos uva ursi* by agar disc diffusion method.

5. Conclusion

Plants are an important source of possibly helpful structures for the creation of novel chemotherapeutic agents. The *in vitro* antibacterial activity assay is the first step toward achieving this objective. The antiviral, antibacterial, antifungal, anthelmintic, and anti-inflammatory qualities of plants have been the subject of numerous reports. A few of these findings have been useful in determining the active ingredient behind these actions as well as in the creation of medications intended for therapeutic use in humans. The results revealed that the aqueous extract of *Arctostaphylos uva ursi* leaf showed significant inhibitory effect against urinary tract infectious agent by using agar disc diffusion method. The strong inhibition was recorded against *S. aureus*. The results of present investigation also interpreted that antibacterial activity of the selected medicinal plant may because of phytochemical constituents. However, it entails further study to know precise mechanism by which the extract exhibits antibacterial activity.

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