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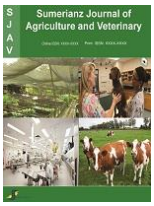

Original Article Open Access

Cichorium intybus: An Excellent Medicinal Herb and Potential Growth Inhibitor of Pathogenic Microorganisms Causing Various Diseases in Humans

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Abstract

Cichorium intybus commonly known as coffee weed in the family Asteraceae is one the famous traditional herb used for curing various human ailments. Recently, different side effects have been reported from the application of artificial antibiotics in human. Hence, the key objective of the current study was to explore the inhibitory potential of *Cichorium intybus*. The ethanolic extracts of flower, leaves, stem and root were prepared for investigating its anti microbial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus atrophaeus*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Citrobacter* and *Candida albicans*. Using well diffusion method, strong activities were observed in terms of inhibition zone against these microorganisms. In current study, best antimicrobial activity (20.3 mm) in leaves extracts was observed against *Klebsiella pneumonia*, 19 mm against *Citrobacter*, 17.5 mm against *Escherichia coli*, 16.3 mm against *Bacillus atrophaeus* and 17.1 mm against *Pseudomonas aeroginosa*. The flower extracts have shown activities of 18.6 mm against *Bacillus subtillus*, 17.3 mm against *Klebsiella pneumonia*, 16.6 mm against *Staphylococcus aureus* and 16 mm against *Escherichia coli*. The root extracts showed an activity of 18.6 mm against *Pseudomonas aeroginosa*, 16.3 mm *against Escherichia coli*, and 15.6 mm against *Klebsiella pneumonia*, *Bacillus subtilis* and *Citrobacter*. The stem extracts showed 16.3 mm activity against *Pseudomonas aeroginosa*, 15.8 mm against *Salmonella typhi*, 15.3 mm against *Candida albicans* (15.3 mm) and 15 mm against *Bacillus subtilis*. The current study exposes the antimicrobial prospective of a medicinally important plant, *Cichorium intybus* as a chief component in possible antibiotic formulation against the given microorganisms and can provide innovative drug discoveries.

Keywords: Cichorium intybus; Pathogenic microorganisms; Antimicrobial activities; Staphylococcus aureus; Candida albicans.

1. Introduction

Since early phases, people were trying to explore the nature of plants for discovering the new drugs. Many valuable medicinal plants have been used for remedial properties to treat numerous diseases [1]. These medicinal plants enormously contribute to the health desires of mankind during the course of their existence. Microorganisms are the major sources of harmful diseases in animals, plants and humans. Various antibiotics are frequently used against these pathogenic microorganisms but nowadays these microbes started resistance to these antibiotics due to mutation. Therefore, the secondary metabolites of plant-based products have the efficiency to fight against virus, bacteria and fungi and also have less toxic and ecofriendly [2]. These secondary metabolites including proven antimicrobial agents are produced in various tissues of medicinal herbs. Thus, the new search and screening of plants should be done to substantiate their use in medicines. *Cichorium intybus* is one of the important medicinal herbs in the family Asteraceae, which is commonly known as chicory and locally it is known as kasani [3]. The plant name was derived from Latin and Greek words, cichorium means field and intybus means to cut, while the Latin word means tubus that indicate the hollow stem [4].

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The *C. intybus* is mostly distributed in Australia, North America, South America, Europe, Africa, and in Asian temperate regions including Pakistan.

Traditionally, the primordial Egyptians cultivate *C. intybus* as a curative and remedial plant [5]. The dried roots were used as additive and substitute of coffee. Its leaves may be used as additive in the vegetables and salad dishes while its extracts is used in invigorating bavereges [6]. Traditionally, it was used for treating diarrhea, for strengthening of reproductive organs, to cure cough, cancer, pulmonary diseases, mild digestive problems and temporary apetite loss [4]. In ancient times, it was used for treating internal infections like sore throat, abdominal cramps, rashes, heamorides, tuberculosis, deafness, melancholy and also as a cathartic for children's [7].

The active extracts of *C. intybus* including oxalic acid, quinic acid, succinic acid and shikimic acid that helps in decreasing biofilm formation and bacterial adhesion to living cells [8]. The seed extracts of *C. intybus* also have effect on some pathogenic microbes like *Staphylococcus aureus, Pseudomonas aeroginosa* and *Escherichia coli.* The root extracts are active against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Micrococcus luteus and Escherichia coli* [9, 10]. The root extracts containing Guaianolides that shows activity against *Trichophyton tonsurans, T. rubrum, anthroprophilic* fungi and *T. voilaceum* [11]. The sesquiterpenoid phytoalexin cichoralexin from *C. intybus* showed anti-fungal properties against *Pseudomonas cichorii* [12]. It is also used for curing various major diseases. The people of Afghanistan are using fresh roots drinks of chicory to relief the fever caused by parasite. The anti-malarial constituents in the chicory are the guaianolide, lactucin and lactucopirin. These constituents completely prevent the HB3 clone of plasmodium (Hondrus-1 strain) when taking its extracts orally at 50meug/ml daily [13]. There is about 95% decrease in the ulcerogenesis when taking extracts of chicory roots orally [14]. The use of chicory seeds extracts reduces alkaline phosphatase, glutamyle pyruvate transaminase and glutamyle oxaloacetate and transaminase serum level and finally protects liver damage [15].

2. Materials and Methods

2.1. Plant Selection

The fresh and healthy plants of *Cichorium intybus* were collected from kanju township, District Swat, Khyber Pukhtoonkhwa, Pakistan. The plants were carefully washed two to three times with tap water to remove the soil and other dust particles. Various parts, flowers, leaves, stem and roots were excised from *C. intybus*. All the fresh parts were weighed for initial fresh biomass and subsequently shade dried for 13 days to obtain dry biomass. After complete drying, all the four parts were grinded, weighed again and used for extraction.

2.2. Test Microorganisms

For investigation of the anti-microbial activity of all the four parts of *C. intybus* (flowers, leaves, stem and roots), Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus and Bacillus atrophaeus*), Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeroginosa and Citrobacter*) and fungi (*Candida albicans*) were applied as a test microorganisms that are mostly causing human diseases.

2.3. Solvent Extracts Preparation

For solvent extraction, 20 ml of pure ethanol was mixed with 3 g powdered material of each part of the *C. intybus* in 100 ml flasks. These flasks were then covered by aluminum foil. Soxhelt extractor was used for getting the best extract in minimum time. After 5 days, extracts were filtered through Whattman paper and the filtrates were collected in 100 ml flasks. To concentrate the filtrate, rotary evaporator was used. The extracts were then dried on water bath and was finally preserved at 4 °C in refrigerator for further use. For anti-microbial assay the stock solution were prepared. Accurately 50 mg extracts of each part was dissolved in 15 ml Dimethyl Sulphoxide (DMSO).

2.4. Preparation of Media

In this study, the nutrient agar media was exploited for antimicrobial assay. Practically, 14 g of nutrient agar powder was added to 500 ml of distilled water and shake well. The media was uniformly mixed using magnetic stirrer and hot plate. The media was then sterilized in autoclave at a pressure 21 psi, temperature 121 °C for 15 minutes. After autoclaving, the media was poured in petri plates using laminar flow hood (LFH), and finally allowed to cool and solidify. The plates were then covered and incubated over night at 37 °C for checking the contamination.

2.5. Anti-Microbial Assay

To find out the antimicrobial activities of *C. Intybus* solvent extracts, well diffusion method was followed on the nutrient agar medium. The media plates were inoculated with inoculums containing 106 CFU/ml of bacteria. After inoculation, wells were made in the center of nutrient agar media with the help of sterile cork borer (5 mm). Exactly, 100 ul of each solvent extract was poured into the wells of inoculated plates. The plates were then covered and incubated for 24 hours at 37 °C. Zones of inhibition around the wells were then measured in mm.

2.6. Data Analysis

Each treatment consists of three replicates. Mean values along with standard deviation were obtained using the statistical software Statistix (v. 8.1; USA). The graphs representing the zones of inhibitions were prepared in OriginLab software (v. 8.5).

3. Results

In this study *C. intybus* viable plants were collected and thoroughly washed with distilled water to remove dust particles. Various parts including flower, leaves, stem and roots were excised weighed separately to determine the fresh weight of flowers (7.75 g), leaves (14.68 g), roots (43.80 g) and stem (30.45 g). These various parts of *C. intybus* were oven dried to determine the dry weight of flower (1.45 g), leaves (2.67 g), roots (11.61 g) and stem (6.53 g) as shown in figure 1. The moisture content was also investigated where flower lost 6.3 g water, leaves 12.1 g, roots 32.19 g and stem lost 23.92 g of water during drying (figure 1). Accurately, 5 g from each part of *C. intybus* was dissolved in 20 ml of ethanol to obtain extractive values. The extractive values of flower was 137 mg, stem was 59 mg, roots was 77 mg and that of leaves were 45 mg as mentioned in figure 2. These crude extracts of various parts were dissolved in dimethyl sulphoxide for extract preparation and antimicrobial activities determination.

The extracts of various parts of *C. intybus* were applied for antimicrobial activities in terms of inhibition zones (Figure 3). The ethanolic extracts of flower showed the best activity against *bacillus subtillus* (zone of inhibition, 18.6 mm) and *Klebsiella pneumonia* (17.3 mm) as shown in figure 4. However, it also showed good activity against *Staphylococcus aureus* (16.6 mm) and *Escherichia coli* (16 mm). The flower extracts has a slight lower activity against *candida albicans* (15 mm), *Citrobacter* (14.6 mm), *Salmonella typhi* (14.5 mm), and *Pseudomonas aeroginosa* (14mm) respectively. The lowest activity was shown against *Bacillus atrophaeus* (12.3 mm) as shown in figure 4. The leaves extracts showed the best activity against *Klebsiella pneumonia* (20.3 mm), *Citrobacter* (19 mm), *Escherichia coli* (17.5 mm) and *Pseudomonas aeroginosa* (17.1 mm) as shown in figure 5. However, moderate activity was shown against *Bacillus atrophaeus* (16.3 mm), *Staphylococcus aureus* (16 mm), and *candida albicans* (15 mm). The lowest activity was recorded against *Bacillus subtilis* (12 mm) and *Salmonella typhi* (10 mm) as shown in figure 5. The stem extracts showed best activity against *Pseudomonas aurogenosa* (16.3 mm) and *Salmonella typhii* (15.8 mm). The extracts also showed a slight lower activity against *Candida albicans* (15.3 mm) and *Bacillus subtilus* (15 mm) as shown in figure 6. However, a moderate activity was recorded against *Citrobactor*, *Escherchia coli*, *Klebsilla pneumonia* (14.3 mm) and *Bacillus atropheus* (14 mm) respectively. Though, the lowest activity of stem extracts was found against *Staphylococcus aureus* as shown in figure 6. The root extracts showed best activity against *Pseudomonas aeroginosa* (18.6 mm). The extracts also showed a slight lower activity against *Escherichia coli* (16.3 mm). However, moderate activity was recorded against *Klebsiella pneumonia*, *Bacillus subtilis* (15.6 mm), *Citrobacter* (15.6 mm) and *Staphylococcus aureus* (15 mm) as shown in figure 7. The lower activity was shown against *Bacillus atrophaeus* (14.6 mm), *Candida albicans* and *Salmonella typhi* (14.3 mm). These results suggest that various parts of C. intybus possess antibiotic like activity against pathogenic microorganisms and can be applied commercially by pharmaceutical industries.

4. Discussion

In the early time fungi, algae, bacteria and plants sources were commonly exploited for extraction of different compounds, which were used in treating many life-threating diseases [16]. Scientists are trying to explore the natural sources of natural antibiotics to combat pathogenic microbes. The purpose of the present study was to find out the antimicrobial potential of *Cichorium intybus* that produce antimicrobial compounds, which are effective against certain pathogenic microbes. Four parts of *Cichorium intybus* were used for determining their antimicrobial activity that is flower, leaves, stem and roots. Almost all parts showed good antimicrobial potential but the best results were achieved with leaves extracts. The extracts of leaves showed antimicrobial activity of 20.3 mm against *Klebsiella pneumonia*, 19 mm against *Citrobacter*, 17.5 mm against *Escherichia coli*, 16.3 mm against *Bacillus atrophaeus* and 17.1 mm against *Pseudomonas aeroginosa*. These results are almost similar to Dulger and Gunuz [17], who recorded the antimicrobial activity of 10 mm in terms of inhibition zone against *Escherichia coli*, 12 mm against *Staphylococcus aureus*, 8 mm against *Klebsiella pneumonia* and 10 mm against *Candida albicans*. In contrast, Dulger and Gunuz [17] do not observe any activity against *Pseudomonas aeroginosa*. Though, in our findings the leaf extracts showed maximum activity against *Pseudomonas aeroginosa*. Furthermore, the discovery of [18] showed the highest activity against *Escherichia coli* having zone of inhibition 35 mm, 34 mm against *Pseudomonas aeroginosa*, 36 mm against *Klebsiella pneumonia* and 53 mm against *Candida albicans* The differences in results may be due to the differences in solvent applied. Farnaz, *et al.* [19], apllied the n. hexane extracts and found no antimicrobial activity in *Chicory*. [20], determine good antimicrobial activities of in leaf extracts of *C. intybus* against fungal species that is *Candida glabrata* and *Candida krusei*. Masood, *et al.* [21], extracted leaves with different solvent but they found no results of leaf extracts in any of the solvents.

Further, the best activity after leaves extracts was observed in flower extracts, which showed an activity of 18.6 mm against *Bacillus subtillus*, 17.3 mm against *Klebsiella pneumonia*, 16.6 mm against *Staphylococcus aureus* and 16 mm against *Escherichia coli*. Mahdieh, *et al.* [22] used the aqueous extract of flower parts on ethylene glycol-induced renal calculi in rats. Though, no other related studies were found on the antimicrobial activities of flower part of *Cichorium intybus* in the literature cited.

The root extracts showed an activity of 18.6 mm against *Pseudomonas aeroginosa*, 16.3 mm against *Escherichia coli*, and 15.6 mm against *Klebsiella pneumonia*, *Bacillus subtilis* and *Citrobacter*. Nandagopal and Ranjitha [10] who conducted a study to determine the phytochemical and anti-bacterial properties of chicory used petroleum ether, hexane, and chloroform solvents for root extraction. They observed 6.8 mm, 5.8 mm, 4.7 mm and 3.5 mm zones of inhibitions against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi* with petroleum ether as a solvent. Using chloroform as a solvent extraction, 4.1 mm, 3.6 mm, 3.0 mm and 5.4 mm zones of inhibitions were recorded against *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*. Using hexane extracts, 12.3 mm, 9.8 mm, 9.6 mm and 7.5 mm

zones of inhibitions were recorded against *B. subtilis, S. aureus, E. coli* and *S. typhi*. These activities are comparatively lower than the present study.

The stem showed least activities as compared to other parts of the *C. intybus*. About 16.3 mm zone of inhibition was recorded against *Pseudomonas aeroginosa*, 15.8 mm against Salmonella *typhi*, 15.3 mm against *Candida albicans* and 15 mm against *Bacillus subtillus*. This is the first experiment in which we applied the stem extracts for antimicrobial activities. No activities were performed for stem extracts in the literature cited.

5. Conclusion

In conclusion, *C. intybus* is one of the potential candidates for extraction of various valuable secondary metabolites that is effective against life-threatening diseases. Comparatively, the leaves portions were found more effective against pathogenic microorganisms than other parts. It means that the leaves of this plant bio-synthesize or accumulate certain secondary metabolites that is potential growth inhibitors and can be extracted for development of new drugs and can be used as best substitute instead of synthetic antibiotics.

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Appendix

Figure-1. Determination of fresh weight, dry weight and moisture contents of flower, leaves, stem and roots of *Cichorium intybus*. Mean values with standard errors are significant when $P<0.01$.

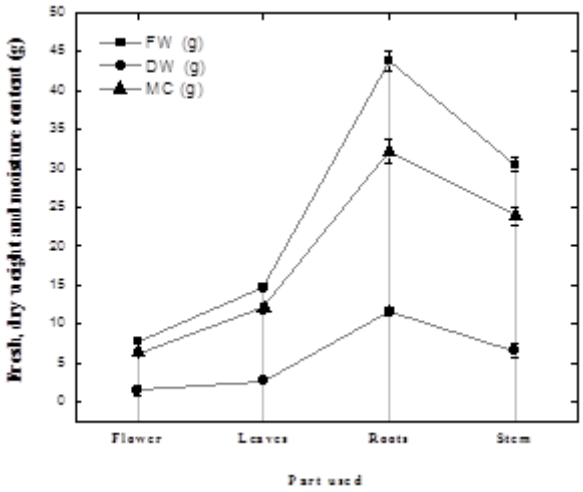

Figure-2. Determination of extractive values of flower, leaves, stem and roots of *Cichorium intybus* in ethanol solvent. Mean values with LSD and ± S.D are significant when $P<0.01$.

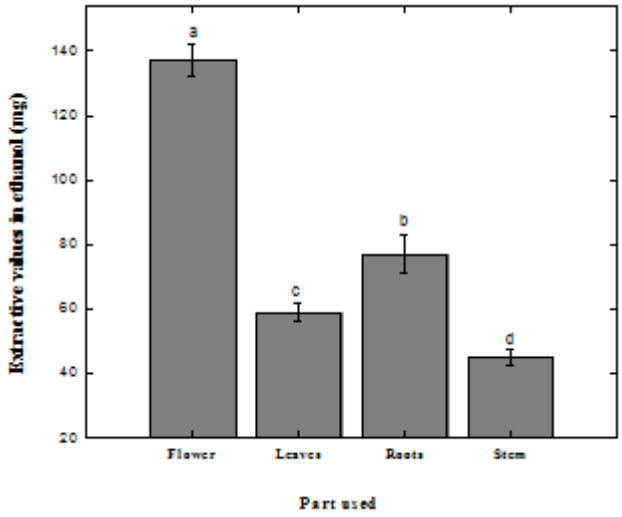

Figure-3. Pictorial presentation of zone of inhibition of **a**. *B. subtilis* (flower), **b**. *K. aeroginosa* (flower), **c**. *C. albicans* (flower), **d**. *E. coli* (flower) **e**. *K. pneumonia* (leaves), **f**. *S. typhi* (leaves), **g**. *C. albicans* (leaves), **h**. *B.*

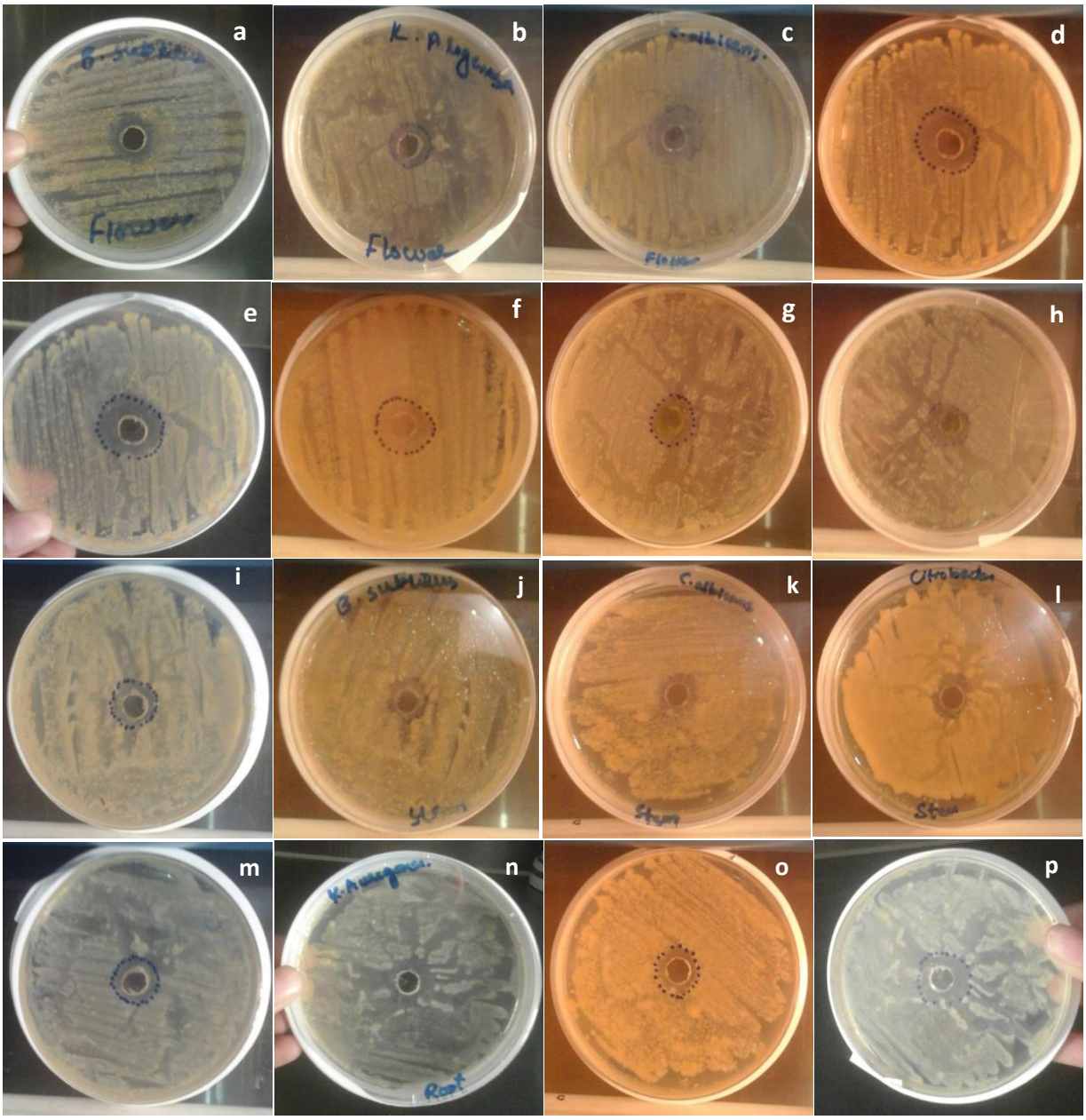

Figure-4. Antimicrobial activities of flower extract of *Cichorium intybus* against pathogenic microorganisms. Mean values with LSD and ± S.D are significant when $P<0.01$.

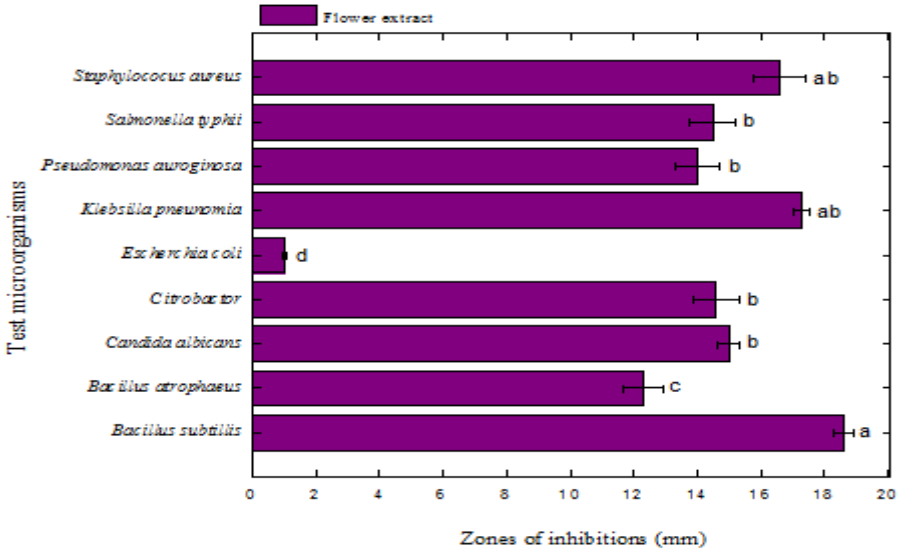

Figure-5. Antimicrobial activities of leaves extract of *Cichorium intybus* against pathogenic microorganisms. Mean values with LSD and ± S.D are significant when *P*<0.01.

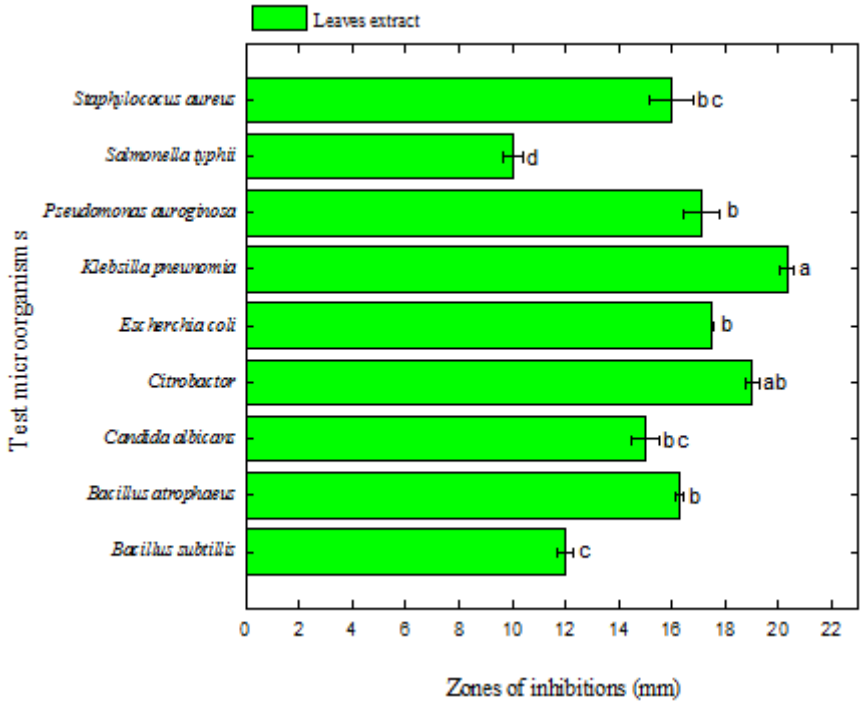

Figure- 6. Antimicrobial activities of stem extract of *Cichorium intybus* against pathogenic microorganisms. Mean values with LSD and ± S.D are significant when *P*<0.01.

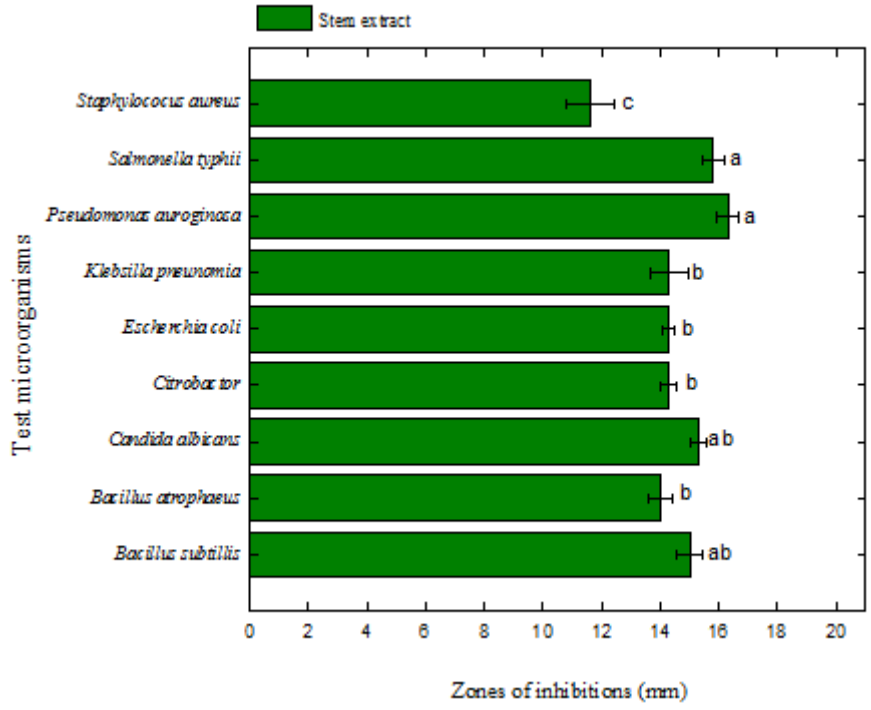

Figure-7. Antimicrobial activities of roots extract of *Cichorium intybus* against pathogenic microorganisms. Mean values with LSD and ± S.D are significant when *P*<0.01