

Full Length Research Paper

In vitro* potential antimicrobial activities of medicinal plants, *Cirsium arvense* and *Cirsium japonicum

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Antimicrobial activities of *Cirsium* species (*Cirsium arvense* and *cirsium japonicum*) was evaluated against six bacterial species, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* (clinical strain/PIMS), *Enterobacter* (clinical strain/PIMS), *Staphylococcus aureus* (MRSA, clinical strain/PIMS) and six fungus *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Candida glabrata* and *Fusarium solani*. The methanolic extracts of these plants were fractionated with *n*-hexane to get (F₁), chloroform (F₂), ethyl acetate(F₃), *n*-butanol(F₄) and methanolic fraction (F₅). The agar well diffusion assay and agar dilution susceptibility testing were carried out to determine the zone of inhibitions and the minimum inhibitory concentration, respectively. Ager well diffusion methods was used for antifungal activity. The zones of inhibition were determined using standard agent. The entire fraction showed some activity against these pathogens, but F₂ of both plants shows very good activity. The present study explains the potential antimicrobial properties of *C. arvense* and *C. japonicum*. From these observations, it is clear that *Cirsium* species contain antimicrobial constituents.

Key words: Traditional medicine, crude extract, antimicrobial activities, *Cirsium arvense*, *cirsium japonicum*.

INTRODUCTION

Throughout the history of mankind, medicinal plants have continuously been used for the treatment of multiple infections (Augustin and Hoch, 2004; Ashraf et al., 2006). Herbal medicines have made large contributions to human healthiness (Iwu et al., 1999) and provided a good source of anti-infective agents; emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections. WHO reported that more than 80% of the world's population depends on traditional medicine for the treatment of their illnesses (Norman et al., 1985). In spite of development of novel drugs in

modern times to combat emerging infections, increased resistance to antibiotics of many bacteria is still a global threat (Konig et al., 2000). This provoked researchers to screen plant extracts and plant compounds for antimicrobial agents (Yoshikazu et al., 2001; Norton, 2000).

Cirsium arvense and *Cirsium japonicum* (L.), *Cirsium* species, family Asteraceae are native plant of Europe, Asia (northern), and widely introduced elsewhere. They are herbaceous plants growing between 30-100 cm, associated with widespread colonies (Morishita, 1999).

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C. arvense is reported nearly in all crops including pastures and rangelands. Likewise, *C. arvense* have a role of natural host to various plants pathogens causing crop spoilage (Parendes and Jones, 2000). Livestock tend to dislike and avoid Canada thistle which may also reduce their consumption of desirable plants in the vicinity of Canada thistle colonies. The crude protein, *in vitro* digestible dry matter, micro and macro mineral concentrations of Canada is comparable to or greater than those of alfalfa (*Medicago sativa*) (Myers, 2000).

The vulnerary use *C. arvense* and *C. japonicum* had not previously been cited in the principal pharmacobotany texts. However, the juice of the leaves of these plants, locally applied, for healing of wounds is used in some parts of the world (Raven and Edwards, 2001; Zouhar, 2001). Fewer reports are available to address the antimicrobial potential of *C. arvense* and *C. japonicum* (Maria et al., 2010). No reports are available on antifungal activities of these plants to the best of our knowledge.

The present study was conducted to evaluate antimicrobial and antifungal activity of *C. arvense* and *C. japonicum* used locally as traditional medicine against multiple infections.

MATERIALS AND METHODS

Plant materials

Collection was based on information given by local inhabitants during follow up of ethno-medical and traditional uses of plant against infectious diseases used locally (Fabricant and Farnsworth, 2001). Plants were identified by Professor Farman Ullah Khan, Department of Botany Bannu, Pakistan. The specimens were deposited and voucher specimen number (117A, 117B) was obtained.

Preparation of extraction

The shade dried plants of *C. species* (11 kg) was ground and extracted with MeOH (35 L×3) at room temperature. The combined methanolic extracts were evaporated under reduced pressure to obtain a dark brown gummy material (600 g). The gummy material was suspended in water and extracted with *n*-hexane (99 g), chloroform (80 g), ethyl acetate (70 g) and *n*-butanol (50 g) soluble fractions, respectively. The fractions were then placed in a vacuum oven at not more than 40°C for about 24 h to remove any residual solvent. These fractions were screened for toxicity. The chloroform and ethyl acetate soluble fractions show highly toxicity against six bacteria and six funguses. The antimicrobial activity was carried out in the Laboratory of Life Science, Quaid-e-azam University, Islamabad, Pakistan. The activity was conformed from School of Science, Beijing University of Chemical Technology.

Preparation of fractions

The plants were cut into pieces and dried at room temperature. The dried plant materials were ground into powder. About 70 g of each plant powdered were dipped in (15LX3) methanol to get dark brown

gummy material of 25 g. Extraction was performed sequentially using *n*-hexane (F₁ 2g), chloroform (F₂ 3g), ethyl acetate (F₃ 4g), *n*-butanol (F₄ 4g), acetone (F₅ 3g) and methanolic (F₆). These methods for the formation of extracts were used for both plants.

Antimicrobial activity

Antibacterial assay

The antibacterial activities were determined using agar well diffusion method (Boakye et al., 1977). Bacterial culture was grown in nutrient broth at 37°C for 18 to 24 h. 0.5 ml of broth culture of test organism was added by sterile pipette into molten agar (50 ml) which were then cooled to 40°C and poured into sterile Petri dish. Sterile cork borer were used to make well of 6 mm in diameter in nutrient agar plate. The wells were filled with given compounds of 100 µl and the plate was left for 1 to 2 h. The plates were incubated at 37°C for 18 to 24 h. Finally, the diameter of inhibition was measured.

Antifungal assay

The antifungal assay was carried out using agar well diffusion method (Hadacek et al., 2000). Sterile dimethyl sulfoxide (DMSO) was used to dissolve the test sample. Sabouraud dextrose agar (SDA) was prepared by mixing Sabouraud 3% glucose agar and agar-agar in distilled water. The required amount of fungal strain was suspended in 2 ml Sabouraud dextrose broth. This suspension was uniformly streaked on Petri plates containing SDA media by means of sterile cotton swab. Compounds were applied into well using same technique for bacteria. These plates were then checked for the presence of zone of inhibitor and result was noted.

Preparation of tested materials

The dilution used for all extracts was 100 mg ml⁻¹. Various extracts of CL were re-dissolved in different solvents whereby, hexane extracts were re-dissolved in hexane. Chloroform was known to be inhibitory to the growth of bacteria. Therefore, the chloroform extracts was re-dissolved in a mixture of petroleum ether and methanol (8:2 v/v).

RESULTS

Almost all the fractions of *C. arvense* and *C. japonicum* showed activity against the bacteria and fungus. The F₂ & F₃ of both species (*C. arvense* and *C. japonicum*) showed very good activity against *Enterobacter*, *Staphylococcus aureus* and *Micrococcus luteus*, while F₁, F₄ and F₅ shows moderate activity against six pathogens (Table 2).

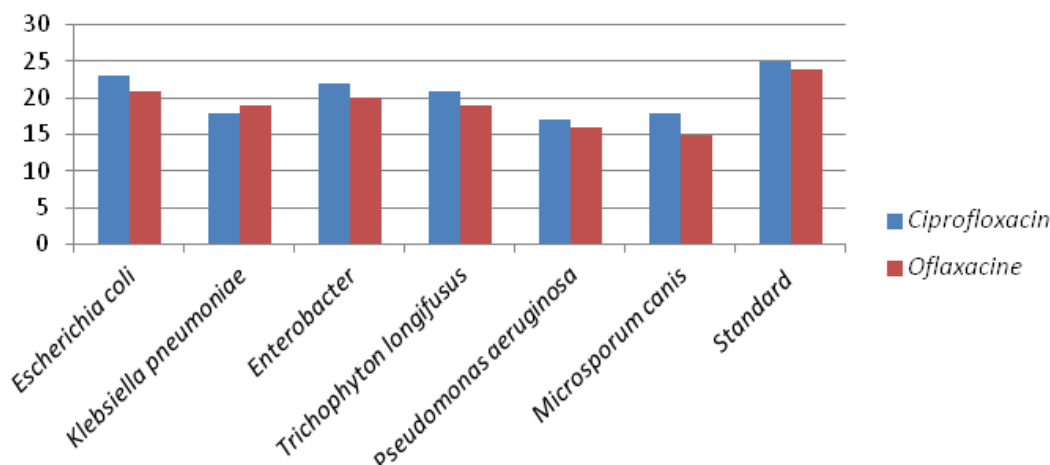
The zone of inhibition of extracts *C. arvense* against six bacteria namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* (clinical strain/PIMS), *Enterobacter* (clinical strain/PIMS), *Staphylococcus aureus* (MRSA, clinical strain/PIMS) and *Micrococcus luteus* (clinical strain/PIMS) were used in antimicrobial assay. Microorganisms were incubated overnight at 37°C in Mueller-Hinton Broth (Oxoid) at pH 7.4. The result is indicated in Table 2 and Figure 2. The

Table 1. Zone of inhibition of reference antibiotics.

Antibiotic	Microorganism						Standard
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter</i>	<i>Trichophyton longifusus</i>	<i>Pseudomonas aeruginosa</i>	<i>Microsporum canis</i>	
Ciprofloxacin	23 (±0.09)	18 (±0.06)	22 (±0.06)	21 (±0.09)	17 (±0.10)	18 (±0.11)	25 (±0.07)
Ofloxacin	21 (±0.8)	19 (±0.10)	20 (±0.05)	19 (±0.06)	16 (±0.12)	15 (±0.11)	24 (±0.18)

Table 2. Zone of inhibition of *C.arvense* plants extracts.

Bacteria	F ₁	F ₂	F ₃	F ₄	F ₅
<i>E. coli</i>	19 (±0.03)	16 (±0.02)	14 (±0.2)	12 (±0.06)	09 (±0.05)
<i>K. pneumoniae</i>	17 (±0.06)	15 (±0.04)	11 (±0.02)	12 (±0.04)	10 (±0.07)
<i>P.aeruginosa</i>	18 (±0.03)	17 (±0.06)	15 (±0.04)	12 (±0.05)	10 (±0.09)
<i>Enterobacter</i>	20 (±0.11)	19 (±0.04)	16 (±0.03)	14 (±0.04)	11 (±0.11)
<i>T. aureus</i>	18 (±0.21)	17 (±0.14)	14 (±0.06)	18 (±0.20)	19 (±0.23)
<i>M. luteus</i>	20 (±0.21)	15 (±0.25)	17 (±0.20)	13 (±0.20)	15 (±0.21)

**Figure 1.** Zone of inhibition of reference antibiotics. Temperature, 37°C; values are inhibition zones (mm) and an average of triplicate. Concentrations used Are 1000 µg/ml.

extracts of these plants were also tested against six fungi, the result of these pathogens is showed in Table 3 and Figure 3. The result of standard drug against these extracts of the plants is summarized in Table 1, Figure 1, Table 4 and Figure 4. While the extracts of *C. japonicum* was also screened against these bacteria and fungus, as mentioned above and the result is indicated in Tables 5 to 7, Figures 5 to 7, respectively.

DISCUSSION

Mostly plants are used as medicine in different parts of the world. The present study compared the extracts of

Cirsium species (*C. arvense* and *C. japonicum*). The whole plant extracts was gotten using different solvents to get different fractions (F₁-F₅). These fractions were used against six bacteria and six fungi. Two reference antibiotic (ciprofloxacin and ofloxacin) were used.

The chloroform and ethyl acetate (F₁ and F₂) of both plants showed good activity. Some researchers have worked on *C. arvense* and isolated some active constituents from chloroform and ethyl acetate fractions (Khan et al., 2011, 2013). The screening of bioactive agent from the plants is one of the most intensive areas of natural products research today. This is the first report that compared the extracts of the *Cirsium* species. There is little difference between the activities of the extracts of *Cirsium*

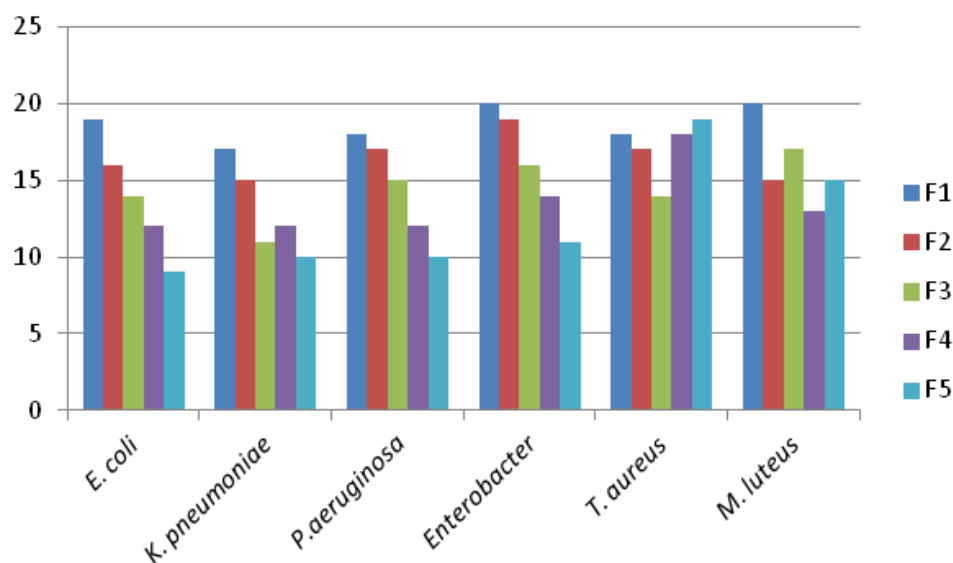


Figure 2. Zone of inhibition of *C. arvensis* plants extracts.

Table 3. Zone of inhabitation of *C. arvensis* extracts of plants.

Fungus	F ₁	F ₂	F ₃	F ₄	F ₅
<i>T. longifusus</i>	18 (±0.20)	14 (±0.02)	11 (±0.25)	10 (±0.50)	10 (±0.08)
<i>C. albicans</i>	19 (±0.32)	17 (±0.07)	13 (±0.12)	15 (±0.21)	12 (±0.70)
<i>A. flavus</i>	17 (±0.25)	15 (±0.20)	16 (±0.24)	18 (±0.07)	14 (±0.90)
<i>M. canis</i>	19 (±0.21)	18 (±0.35)	19 (±0.23)	16 (±0.08)	16 (±0.20)
<i>C. glaberata</i>	20 (±0.17)	18 (±0.21)	12 (±0.60)	19 (±0.05)	15 (±0.25)
<i>F. solani</i>	19 (±0.06)	17 (±0.02)	13 (±0.40)	11 (±0.21)	17 (±0.18)

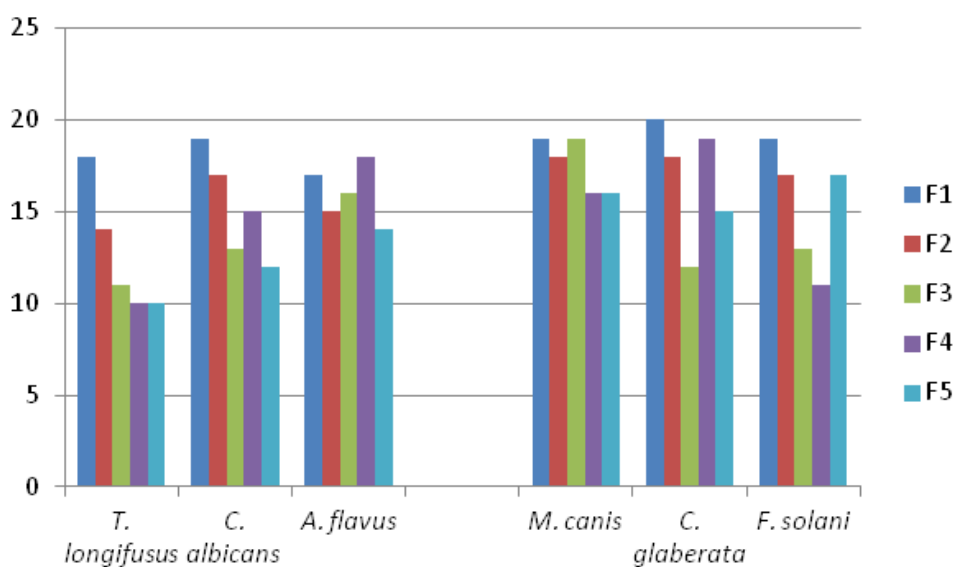
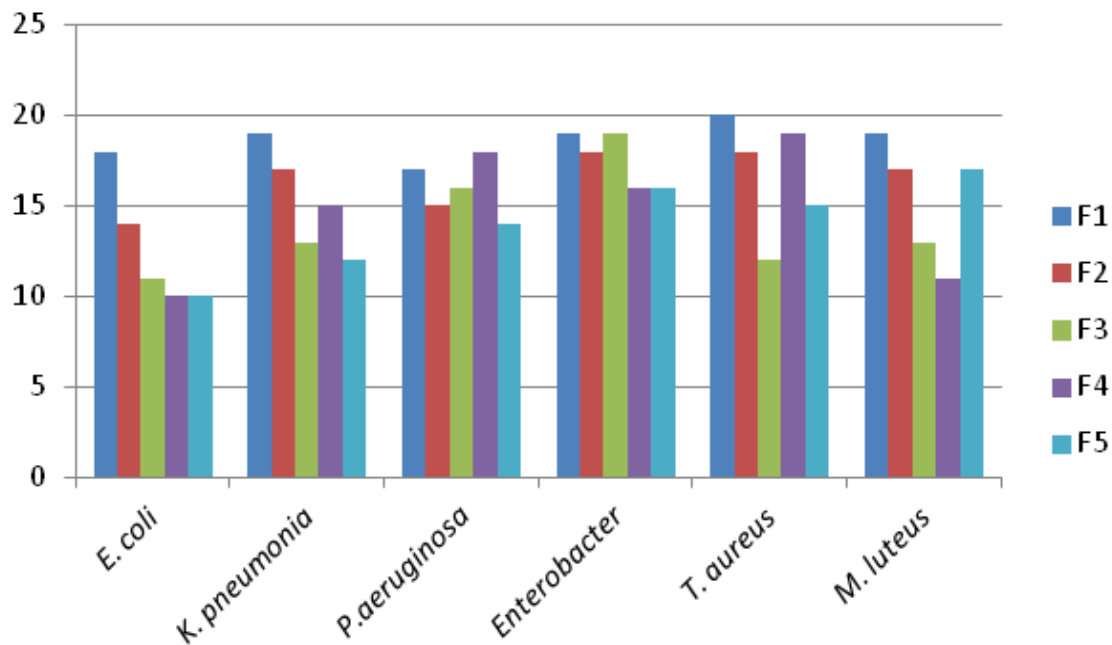


Figure 3. Zone of inhabitation of *C. arvensis* extracts of plants.

Table 4. Zone of inhibition of *C. japonicum* plants extracts.

Bacteria	F ₁	F ₂	F ₃	F ₄	F ₅
<i>E. coli</i>	18 (±0.20)	14 (±0.02)	11 (±0.25)	10 (±0.50)	10 (±0.08)
<i>K. pneumonia</i>	19 (±0.32)	17 (±0.07)	13 (±0.12)	15 (±0.21)	12 (±0.70)
<i>P. aeruginosa</i>	17 (±0.25)	15 (±0.20)	16 (±0.24)	18 (±0.07)	14 (±0.90)
<i>Enterobacter</i>	19 (±0.21)	18 (±0.35)	19 (±0.23)	16 (±0.08)	16 (±0.20)
<i>T. aureus</i>	20 (±0.17)	18 (±0.21)	12 (±0.60)	19 (±0.05)	15 (±0.25)
<i>M. luteus</i>	19 (±0.06)	17 (±0.02)	13 (±0.40)	11 (±0.21)	17 (±0.18)

**Figure 4.** Zone of inhibition of *C. japonicum* plants extracts.**Table 5.** Zone of inhibition of reference antibiotics.

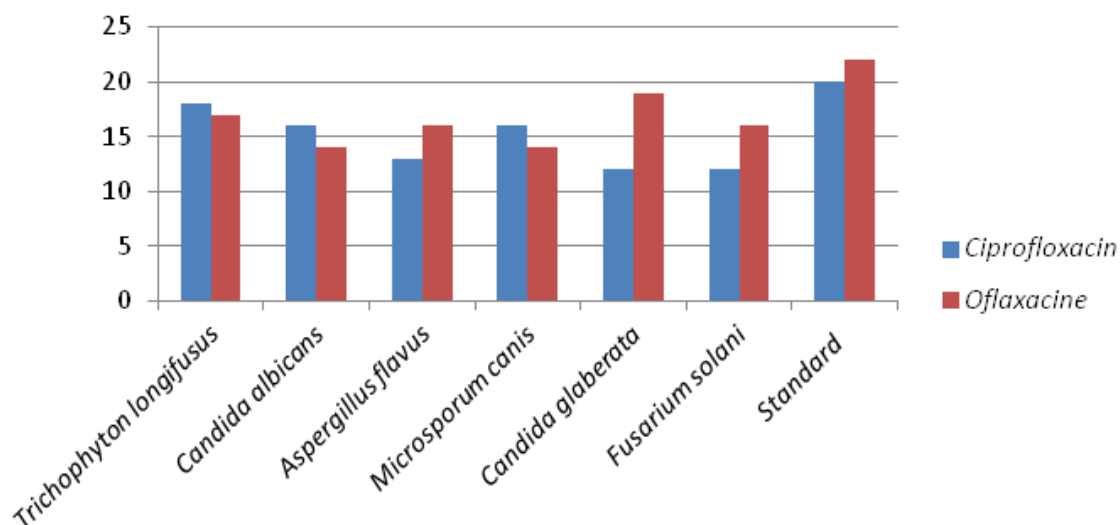
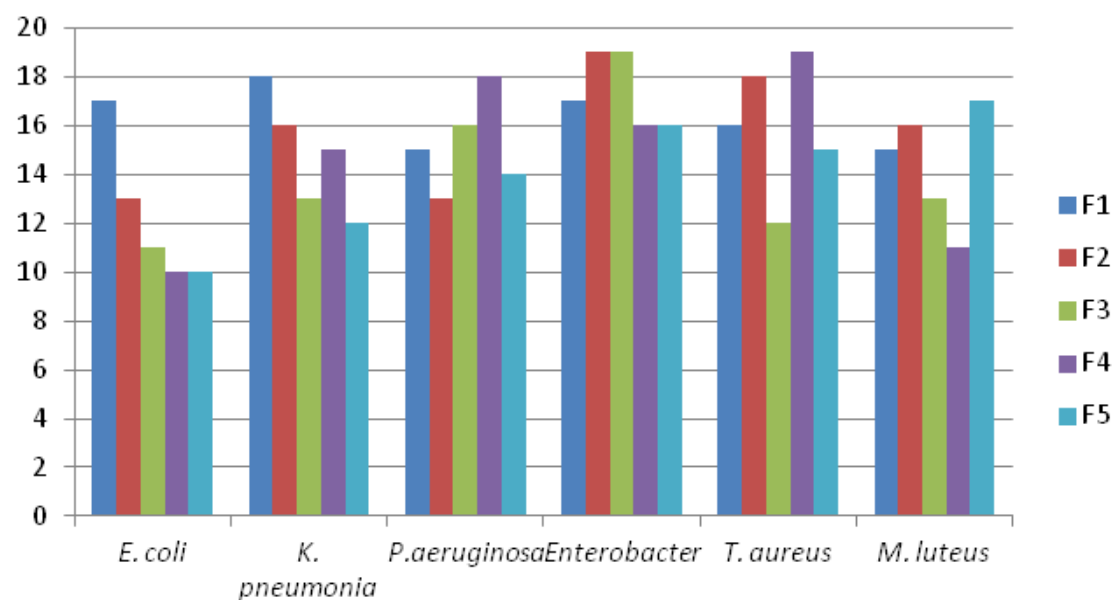
Antibiotic	Microorganism						Standard
	<i>Trichophyton longifusus</i>	<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Microsporium canis</i>	<i>Candida glabrata</i>	<i>Fusarium solani</i>	
Ciprofloxacin	18 (±0.09)	16 (±0.80)	13 (±0.08)	16 (±0.10)	12 (±0.09)	12 (±0.32)	20 (±0.5)
Oflaxacine	17 (±0.8)	14 (±0.70)	16 (±0.09)	14 (±0.08)	19 (±0.02)	16 (±0.09)	22 (±0.19)

Table 6. Zone of inhibition *C. japonicum* plants extracts.

Bacteria	F ₁	F ₂	F ₃	F ₄	F ₅
<i>E. coli</i>	17 (±0.34)	13 (±0.09)	11 (±0.20)	10 (±0.59)	10 (±0.10)
<i>K. pneumonia</i>	18 (±0.22)	16 (±0.03)	13 (±0.17)	15 (±0.25)	12 (±0.74)
<i>P. aeruginosa</i>	15 (±0.19)	13 (±0.22)	16 (±0.23)	18 (±0.98)	14 (±0.90)
<i>Enterobacter</i>	17 (±0.19)	19 (±0.33)	19 (±0.20)	16 (±0.28)	16 (±0.20)
<i>T. aureus</i>	16 (±0.21)	18 (±0.20)	12 (±0.43)	19 (±0.35)	15 (±0.25)
<i>M. luteus</i>	15 (±0.06)	16 (±0.05)	13 (±0.40)	11 (±0.51)	17 (±0.18)

Table 7. Zone of inhibition of *Cirsium japonicum* extracts.

Fungus	F ₁	F ₂	F ₃	F ₄	F ₅
<i>T. longifusus</i>	17(±0.18)	15(±0.10)	13(±0.20)	12(±0.14)	15(±0.10)
<i>C. albicans</i>	18(±0.20)	14(±0.20)	12(±0.10)	16(±0.14)	14(±0.60)
<i>A. flavus</i>	16(±0.20)	13(±0.22)	14(±0.09)	19(±0.18)	13(±0.90)
<i>M. canis</i>	17(±0.09)	16(±0.23)	17(±0.02)	16(±0.18)	12(±0.20)
<i>C. glaberata</i>	19(±0.12)	17(±0.25)	13(±0.08)	20(±0.09)	17(±0.25)
<i>F. solani</i>	17(±0.29)	14(±0.20)	10(±0.50)	13(±0.21)	18(±0.18)

**Figure 5.** Zone of inhibition of reference antibiotics.**Figure 6.** Zone of inhibition *C. japonicum* plants extracts.

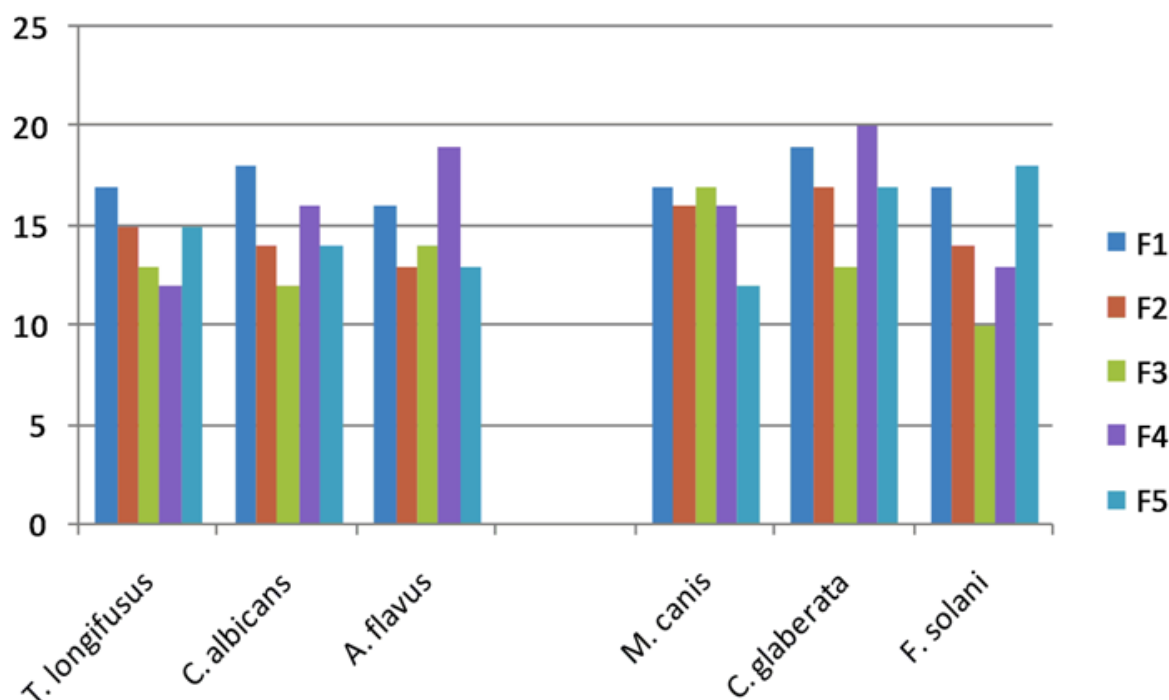


Figure 7. Zone of inhibition of *Cirsium japonicum* plants extracts.

species. The F₃, F₄ and F₅ of both plants extracts showed moderate activity against six pathogens.

The plants extracts showed good antifungal activity. Like bacteria, F₂ and F₃ showed good antifungal activity. F₄ of both medicinal plants show very good antifungal activity. While fraction F₅ showed moderate activity. This was comparatively less than the reports of a recently published research (Maria et al., 2010).

This variation may be due to geographical location of plant. The present study shows that *Cirsium* species have potential antimicrobial activity and further study have been recommended to show the medical properties of these species.

Conclusion

The result shows that *Cirsium* species contain active ingredients against bacteria and fungus. Several antimicrobial activity constituents have been isolated from these plants; these plants are used as folk-medicine in different parts of the world. From this, it is clear that *Cirsium* species have potential antimicrobial activity against different pathogens. Several flavonoid and phenolic compounds have been isolated from these genera. We highly recommend the family species for further research to exploit the hidden medicinal properties of these plants, to best of our knowledge; it will contain highly active alkaloids.

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