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Comparison of Biological Potential between Aqueous Extracts of *Rumex crispus* Leaf and Root as Antibacterial, Synergistic Agents: A TLC-Bioautography and Cytotoxic Study

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SUMMARY

The present study deals with the evaluation of the comparison between the aqueous leaf (RCL) and root extract of *Rumex crispus* (RCR). *In-vitro* analysis such as antibacterial, sensitivity and cytotoxic effects and TLC-bioautography against bacterial pathogens of both aqueous leaf (RCL) and root extract of *Rumex crispus (RCR)* was carried out. Highly resistant microbes were developed, which are the major concern these days. Extracts were prepared by the maceration technique. The antibacterial activity, sensitivity testing, cytotoxic assay, thin layer chromatography, and TLC-bioautography were carried out via the agar well diffusion method, disc diffusion method, MTT assay, and agar overlay technique, respectively. The antibacterial effect showed that RCL (*Rumex crispus* leaf extract) had much more potential against bacterial pathogens when compared to RCR (*Rumex crispus* root extract) at all the concentrations used (5 mg/ml, 10 mg/ml, and 15 mg/ml). The inhibition potential increased with increased concentration. Sensitivity testing showed that amikacin and ciprofloxacin impregnated with extract had more inhibitory potential, but RCL extract showed more effective results when compared to RCR extract. The MTT assay showed that a significant reduction of bacterial cells was found due to RCL and RCR. RCL showed the maximum cytotoxic effect when compared to RCR. TLC profiling and phytochemical screening showed the presence of important phytoconstituents like antioxidants, glycosides, alkaloids, proteins, saponins, and flavonoids. TLC bioautography supported the findings of the antibacterial assay. It was concluded that RCL extracts with different concentrations had a greater potential effect than RCR extract.

Keywords: *Rumex crispus*, antibacterial activity, sensitivity testing, antioxidant activity

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INTRODUCTION

People have utilized plant-based herbal treatments for a long time. Even now, these plants are attracting a lot of attention as experts investigate what they can do for

human health. They've discovered some pretty good drugs that can help with critical conditions by studying these plants. These medications were found by scientists examining specific plants and what they can do for human health (Lonkala and Reddy, 2019; Lonkala and Reddy, 2019; Marasini *et al.*, 2020; Marasini *et al.*, 2020; Pantha *et al.*, 2020; Bhandari *et al.*, 2021).

Scientists are investigating chemicals derived from medicinal plants in order to discover new strategies to combat hazardous microorganisms. This review looks at how these plant compounds can kill bacteria, how they work, and how they can be used to make useful chemicals. It also discusses the limitations and potential prospects of employing plant-based chemicals to combat germs. Utilizing natural substances from medicinal plants to fight germs is difficult, but it is gaining popularity among researchers. Even though several countries have approved synthetic medications to combat germs, scientists are still interested in natural chemicals derived from plants. The goal is to discover new ways to fight germs without using as many antibiotics, as well as to address the problem of bacteria becoming resistant to present therapies. As a result, researchers have discovered and identified novel active compounds derived from plants that can aid in the fight against bacterial resistance (Vaou *et al.*, 2021; Moloney, 2016; Tortorella *et al.*, 2019; Vaou *et al.*, 2021).

Natural antimicrobial compounds can be used alone or in conjunction with medicines to combat a wide range of bacteria. Some of these natural substances have the ability to destroy germs while also assisting antibiotics in their effectiveness. Even if they cannot kill bacteria on their own, some of them can be useful when combined with antibiotics to combat antibiotic resistance in bacteria. Because they have fewer adverse effects than synthetic medications, these complex natural molecules have the potential to be useful in the treatment of disorders. They also do not allow bacteria to become resistant as quickly (Ody, 2017; Galeane *et al.*, 2017; Enan *et al.*, 2020; Fazly-Bazzaz *et al.*, 2018; Ruddaraju *et al.*, 2020; Poddar *et al.*, 2020; Adeleye *et al.*, 2021; Akhtar, 2021; Nazir, 2021).

Pathogens and parasites resistant to numerous medications are a major issue. They pose a significant threat to our public health system. To address this, we require innovative drugs that treat these diseases in novel ways. On the other hand, there is a large body of scientific research demonstrating that medicinal plants can effectively heal ailments. Thus, exploiting plants as a source for new medications could be a novel and successful approach to combating drug resistance (Vasas *et al.*, 2015; Idris *et al.*, 2017; Bello *et al.*, 2019; Idris *et al.*, 2019). Researchers discovered saponins, tannins, flavonoids, essential oils, and anthraquinone derivatives such as chrysophanol and emodin in *R. crispus* (See Jang *et al.*, 2018 and Prateeksha *et al.*, 2019).

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

All the chemicals and solvents used in this experiment were analytical grade with ≥ 95% purity were obtained from CARLO ERBA (Itlay), Bioworld (USA), Bioanalyse Turkey and Sigma Aldrich (Germany). Apparatus used were Weighing balance (SHIMADZU Japan), Autoclave (Sturdy Apex Taiwan), thermostat incubator with

shaker (ZHP-100 China), Hot plate and centrifuge (SCILOGEX China), UV-Vis Spectrophotometer (Pg instruments USA), Laminar Flow (ESCO world class Singapore), Micropipettes (Milward UK).

PLANT MATERIALS

In Himalayan region (Altaf and Umair, 2022), *Rumex crispus* was collected from the Qandeel colony in Bagh, AJK, Pakistan. The plant was identified by an ethnobotanist from the Department of Botany at the Women's University of AJ&K in Bagh. They washed the entire plant with tap water to remove dirt before drying it in the shade at room temperature (about 20°C, plus or minus 2 degrees). They pounded the plant into a fine powder once it had completely dried.

EXTRACT PREPARATION

They employed a procedure called maceration to extract extracts from *Rumex crispus* leaves and roots, as described by Azwanida in 2015. For each extract, they blended 10 grams of finely powdered plant material with 100 milliliters of distilled water, one for the leaves and one for the roots (Figure 1).

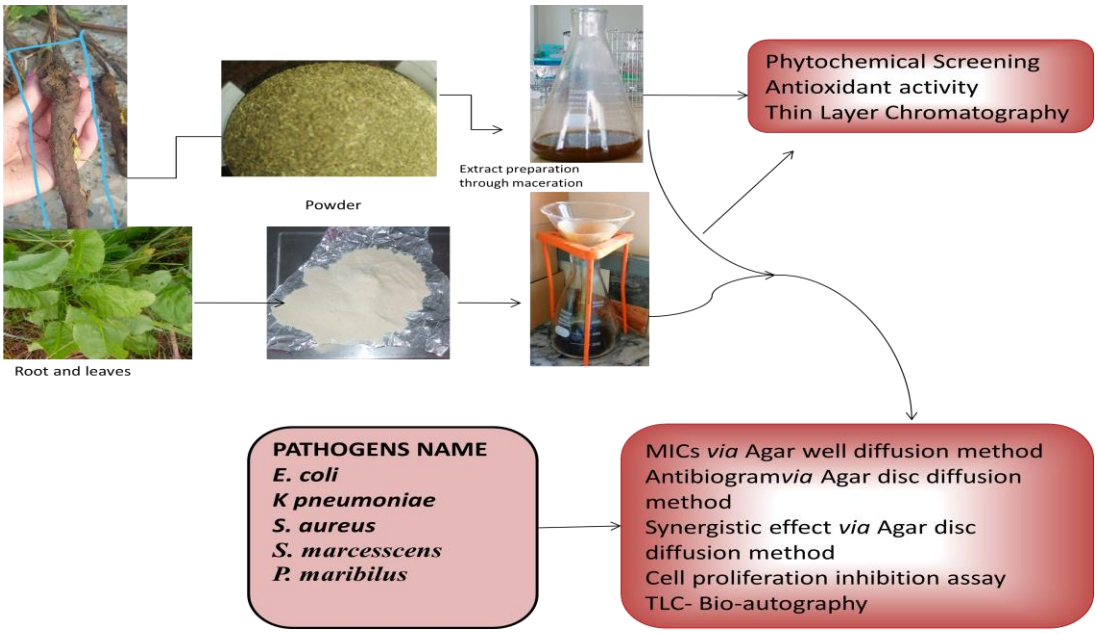


Figure: 1: Scheme of study for the research.

BACTERIAL PATHOGENS

The antibacterial activity of was assessed against four Gram negative (*Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*) and one Gram positive (*Streptococcus pyogenes*) bacterial pathogens, isolated from various clinical samples.

AGAR WELL DIFFUSION METHOD

Agar well diffusion method was used to evaluate the antibacterial activity (Balouiri *et al.*, 2016) the agar plate surface is inoculated by spreading a volume of the microbial in-oculum over the entire agar surface. Three wells with a diameter

5 mm were punched aseptically with sterile yellow tips then sterilized needles were used to remove agar plug. About 30 µl of RCR, RCL, ciprofloxacin 10 µg (control) were poured in every prepared well and then placed in an incubator for 24 h at 37ºC. The diameter of the zone of inhibition in millimeter (mm) was measured after 24 h (Seeley and Van Demark, 1962) Tests were performed in triplicates (Smânia*et al.*, 1999).

SENSITIVITY TEST

They sought to test how well extracts from *Rumex crispus* roots (RCR) and leaves (RCL) worked with antibiotics in an experiment. They used the agar disc diffusion method, as described by Balouiri *et al.* 2016. They immersed tiny antibiotic-containing discs (amikacin and ciprofloxacin) in each extract. After drying the discs under a clean air flow, they evenly spread three of these discs with each extract onto the surface of the agar in a Petri plate. After that, the Petri plates were stored at 35°C for 24 hours. Following that, they measured and compared the areas where the bacteria couldn't grow, known as the "zone of inhibition." This enabled them to assess how well the extracts and antibiotics interacted.

THIN LAYER CHROMATOGRAPHY

TLC (Thin-Layer Chromatography) with Silica gel 60F254 plates was used to confirm phyto-constituents, as described by Akhtar and Ali, 2022. For the screening, two buffer systems, BS1 and BS2, were used. BS1 was made up of a precise ratio of Chloroform, Ethyl acetate, and Acetic acid, as well as Acetic acid and Distilled water. Chloroform, Acetone, and Distilled water were used to make BS2. The TLC plates were viewed under visible light after the samples were applied and allowed to dry, and the spots were seen and recorded. The retention factor (Rf) for each spot was calculated by the researchers to examine the separation of these spots. This factor quantifies how far the chemical moved on the plate by comparing it to the distance traveled by the solvent.

AGAR OVERLAY BIOASSAY

Following the procedure outlined by Dewanjee *et al.* in 2015, a direct bioautography method was used with minor adjustments to test against various bacterial pathogens, including *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, and Proteus mirabilis*. RCR and RCL extracts were utilized to create chromatograms on pre-coated TLC Silica gel 60F254 plates. The generated TLC plates were then sprayed with DPPH (2,2-diphenyl-1-picrylhydrazyl). After that, the plates were placed in sterile Petri dishes and bacterial cultures were poured on top. The Petri dishes were maintained at room temperature until solidification under a clean air flow, and then incubated overnight at 37°C. The following day, the zones of inhibition around each location on the chromatograms were inspected and recorded.

CELL VIABILITY ASSAY

Cytotoxicity evaluation using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) was used to estimate the viability of the bacterial cells (Gerlier and Thomas set, 1986) the human bacterial pathogens were cultured in freshly

prepared nutrient broth medium (4ml) at 37ºC for overnight. The next day, overnight bacterial cell culture (100 µl) was poured in freshly prepared nutrient broth medium (1 ml) along with 100 µl of each sample in triplicate with control and blank (*Rumex crispus* leaf and root extract separately RCL and RCR, Amikacin) then incubated at 37ºC for 4 h. For the reduction reaction, 10 µl of MTT was added and the mixture was incubated at 37ºC (without shaking) for 2-4 h with an opened tube cap. The formation of formazan crystals during this reaction (observed purple color) were observed, and DMSO (500 µl) was added. The absorbance was recorded at 570 nm through a spectrophotometer. DMSO was taken as a control.

RESULTS

Antibacterial activity showed that RCL (*Rumex crispus* leaf aqeous extract) was more toxic to five tested bacterial pathogens as compared to RCR (*Rumex crispus* root aqueous extract) as shown in table 1 and figure 2. RCL showed potential inhibition effect at all concentrations (5 mg/ml, 10 mg/ml and 15 mg/ml) except 5 mg/ml which showed least antibacterial activity (Table 1). Maximum zones of inhibitions were recorded at 15 mg/ml, inhibition effect increases as the concentration increases. RCL showed highest activity (16 ± 0.81 mm, 12± 8.81 mm and 12± 1.63mm) against *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli* at the concentration of 15 mg/ml. RCR showed highest zones 13.6 ± 1.9 mm and 11 ± 0.8 mm against *Streptococcus pyogenes* and *Klebsiella pneumonia* respectively and moderate against *Pseudomonas aeruginosa, Proteus mirabilis* and *Escherichia coli* (8.6±2.6 mm, 8 ± 1.63mm and 6 ±0.81mm). Moderate inhibition was seen at the concentration of 10 mg/ml RCR against all tested pathogens where as 5 mg/ml of RCL, 5 mg/ml RCL and 10 mg/ml showed low antibacterial activity as shown in table 1. DMSO had no effect on the growth of tested pathogens.

Table 1: Antibacterial activity of RCL and RCR against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.*

Pathogens	***Rumex crispus* leaves extract (RCL) at different conc.**			***Rumex crispus* root extract (RCR) at different conc.**		
	5mg/ml	**10g/ml**	**15g/ml**	**5g/ml**	**10g/ml**	**15g/ml**
Klebsiella pneumonia	8.33 ±0.47	15.3± 0.47	16 ± 0.81	3.3 ±0.47	7.33 ± 0.47	11 ± 0.81
Streptococcus pyogenes	1.66± 0.471	0.33± 1.24	5.33± 1.69	7± 1.63	7 ± 1.41	13.6 ± 1.9
Escherichia coli	2 ± 0.81	1.66 ± 0.47	12± 1.63	1.3± 0.47	3 ±0.81	6 ±0.81
Pseudomonas aeruginosa	2.66± 0.94	3.33 ± 0.47	12± 8.81	1.3± 0.47	6.33 ± 0.47	8.6± 2.62
Proteus mirabilis	8 ± 1.41	8 ± 1.41	9 ±1.41	2.3± 0.47	5.33 ± 1.24	8 ± 1.63

Sensitivity test was further done against all tested pathogens (*Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*) at all concentrations 5 mg/ml, 10 mg/ml and 15 mg/ml (as shown

in table 2, table 3 and figure 3 and 4). Ciprofloxacin (in combination with RCL at 15 mg/ml) showed maximum zones of inhibition against *Escherichia coli* (33 ± 0), *Pseudomonas aeruginosa* (19 ± 1.41), *Klebsiella pneumonia* (18.6 ± 0.94) and effective results were recorded against *Proteus mirabilis* (14.6± 0.47). Approximately same results were observed at 10 mg/ml (ciprofloxacin+RCL) against *Escherichia coli* (33± 0 mm), *Klebsiella pneumonia* (17.6± 1.69), *Streptococcus pyogenes* (16.3 ± 1.69 mm) and *Proteus mirabilis* (14.6± 0.47 mm) whereas *Pseudomonas aeruginosa* also showed effective result (13.6 ±2.35 mm). In case of RCR, ciprofloxacin showed effective inhibitions at both 10 mg/ml and 15 mg/ml against all pathogens where as moderate zones were recorded at 5 mg/ml (Table 2). Strong inhibition of *Escherichia coli* (19 ± 1.41 mm and 19.6 ±0.88 mm), *Pseudomonas aeruginosa* (16.3± 0.47 mm and 17.6 ±0.47 mm), *Streptococcus pyogenes* and *Proteus mirabilis* (16 ± 0.81 mm and 16 ± 0.81 mm) were recorded at (10 mg/ml and 15 mg/ml), concentrations of ciprofloxacin+RCR. As the concentration increased inhibition rate also increased (as shown figure 3).

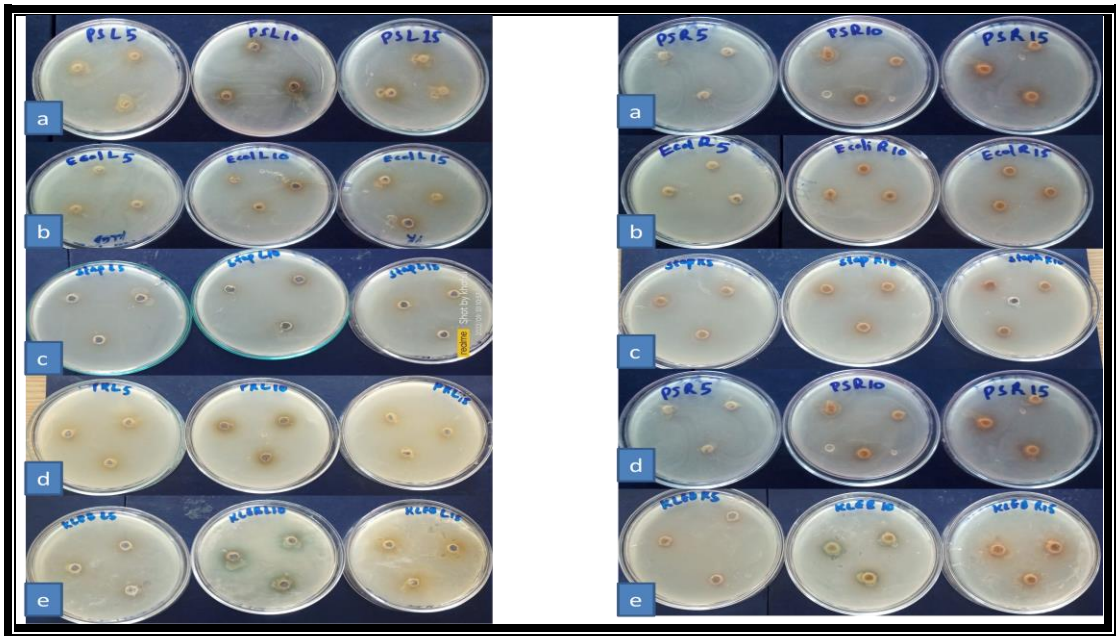


Figure 2: Antibacterial activity of root (RCR) and leaf extract (RCL) of *Rumex crispus* against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.*

Amikacin (in combination with RCL at 5 mg/ml, 10 mg/ml and 15 mg/ml) showed maximum zones of inhibition against *Escherichia coli*(23± 2.16 mm at 15 mg/ml), *Klebsiella pneumonia* (22.6 ± 2.16 mm at 15 mg/ml) and *Proteus mirabilis* (19 ± 0.81 mmat 15 mg/ml) and effective results were recorded against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. In case of RCR *Streptococcus pyogenes, Klebsiella pneumonia* and *Pseudomonas aeruginosa* showed maximum zones of inhibition at the concentration of 10 mg/ml and 15 mg/ml (as shown in table 3 and figure 4).

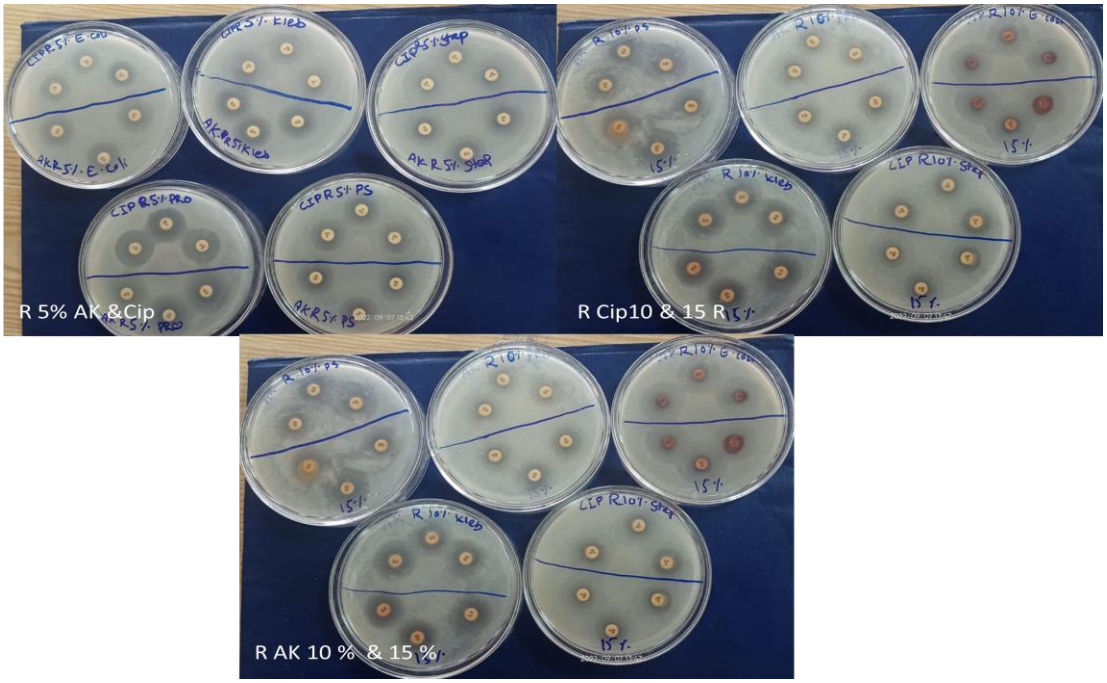


Figure: 3. Sensitivity effect of antibiotics (AK and Cip) and *Rumex crispus* root extract against tested bacterial pathogens (*Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*).

Table 2: Sensitivity test of extracts (RCL and RCR) and Ciprofloxacin (CIP) against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.*

Pathogens	*Rumex crispus* leaves extract + CIP			*Rumex crispus* roots extract +CIP			Control
	5 mg/ml RCL+CIP	10 mg/ml RCL+CIP	15 mg/mlRCL +CIP	5 mg/mlRC R+CIP	10 mg /ml RCR+CIP	15 mg/mlRCR +CIP	CIP (30 µg)
Klebsiella pneumonia	17 ± 2.44	17.6± 1.69	18.6 ± 0.94	9.66 ± 1.2	15.6 ± 0.94	15.6 ± 1.24	15±0.81
Streptococcus pyogenes	15.6 ± 1.24	16.3 ± 1.69	17.6 ± 0.47	16.6 ± 1.2	16.3 ± 1.24	16 ± 0.81	16 ± 0.81
Escherichia coli	18.3± 0.47	33± 0	33 ± 0	5.3 ± 1.24	16.6 ± 2.36	19 ± 1.41	19.6 ±0.88
Pseudomonas aeruginosa	7.6± 1.69	13.6 ±2.35	19 ± 1.41	19 ± 1.41	16.3 ± 1.69	16.3± 0.47	17.6 ±0.47
Proteus mirabilis	12 ± 0.81	13± 0.81	14.6± 0.47	14.6 0.47	14.6 ± 0.47	16 ± 0.81	16 ± 0.81

RCL and RCR TLC profiling give effective result and showed the presence of various phytochemicals. Different phytoconstituents had different Rf values in two solvent systems. Brown and yellow spots on slica gel coated plates had 0.7 and 0.8 in solvent system 1 where as 0.9 and 0.87 in solvent system 2 shown in table 4.

Table 3: Sensitivity test of extracts (RCL and RCR) and Amikacin (AK) against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.*

Pathogens	*Rumex crispus* leaves extract + AK			*Rumex crispus* roots extract + AK			Control
	5g RCL+ AK	10g RCL+AK	15gRCL+ AK	5g RCR+ AK	10g RCR+ AK	15gRCR+ AK	AK
Klebsiella pneumonia	20.3 ± 0.47	20.6 ± 0.94	22 ± 2.16	9.66 1.24	15.6 ± 0.94	20.6 ±1.24	12 ± 0.81
Streptococcus pyogenes	12.66 ± 0.94	17 ± 0.81	17 ± 0.81	16.6± 1.24	17.3 ± 1.24	21 ± 0.81	16 ± 0.81
Escherichia coli	22 ± 2.49	23 ± 2.16	23 ± 2.16	5.33 ± 1.24	16.6 ± 2.36	19 ± 1.41	12.6 ± 0.9
Pseudomonas aeruginosa	11 ± 0.81	11.3± 1.24	13.6 ± 1.24	19 ± 1.41	20.3 ± 1.69	20.3 ±0.47	17.6 ± 0.5
Proteus mirabilis	14.6 ± 0.94	18 ± 0.81	19 ± 0.81	14.6± 0.47	14.6 ±.47	16 ± 0.81	12 ± 0.81

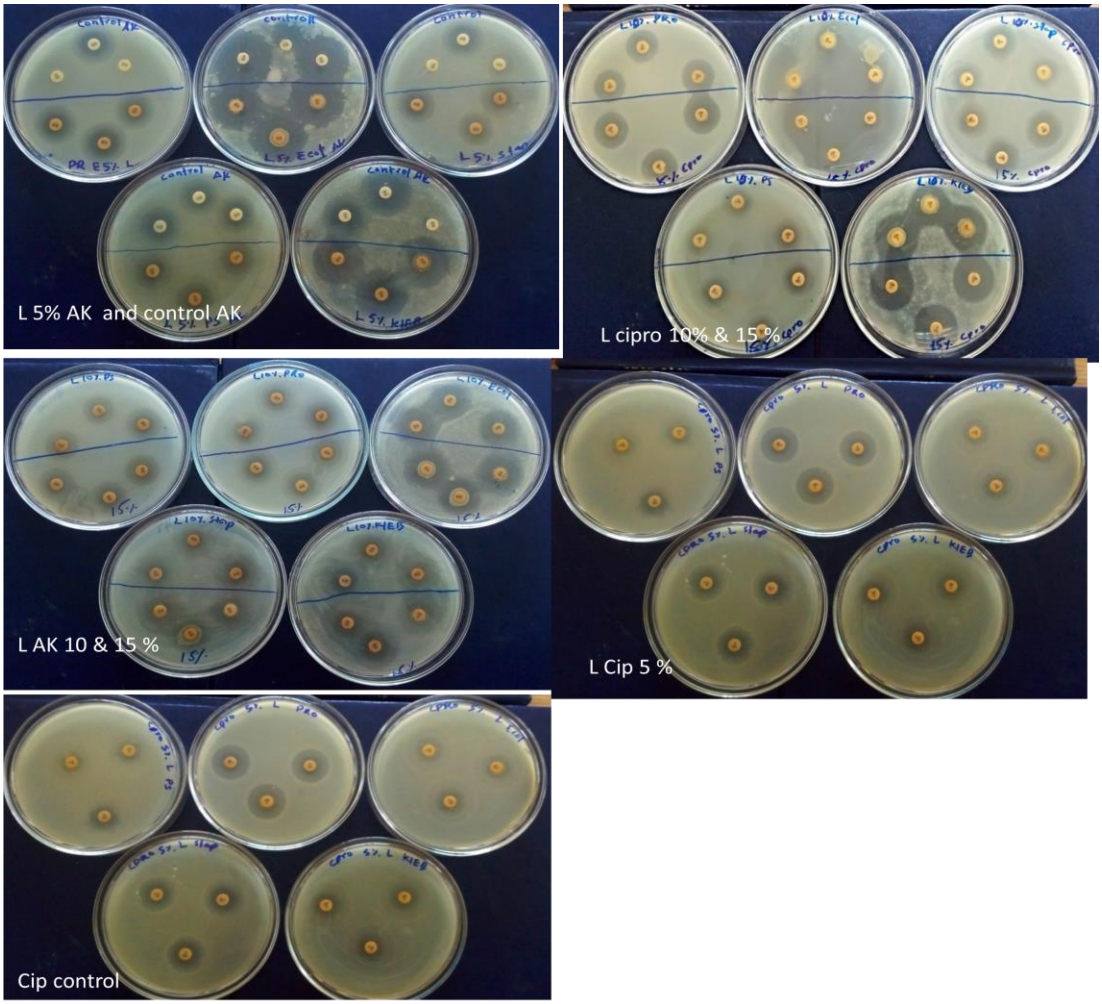


Figure 4: Sensitivity test of antibiotics (AK and Cip) and Leaf extract of *Rumex crispus* against tested bacterial pathogens (*Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*).

Phyto-constituents of RCL and RCR were separated on TLC plates (shown in figure 5). TLC plates analysed for phytoconstituent screening showed the presence of antioxidants and Amino acids. After sprayed with DPPH yellow color spots were observed which confirmed the presence of antioxidants and after spraying ninhydrin solution reddish brown spots were observed which confirmed the presence of amino acid shown in figure 5.

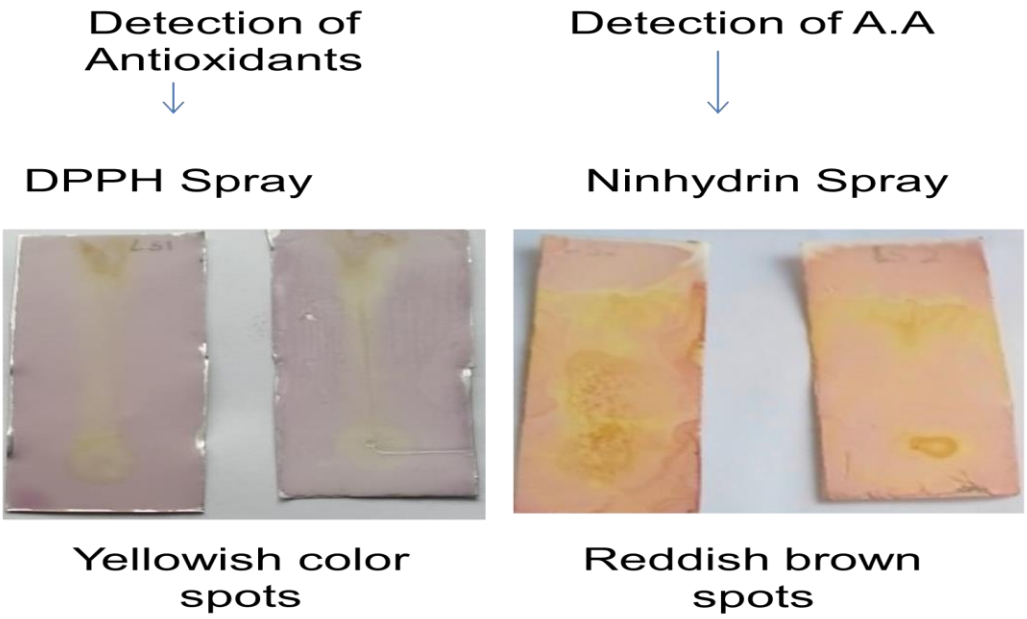


Figure 5: Thin layer chromatography of aqueous extract of *Rumex crispus.*

Qualitative screening of phytoconstituents (shown in figure 6) through different protocols showed the presence of glycosides, alkaloids, proteins, saponins and flavanoids. This was confirmed from the different color changes depicted by individual compounds when subjected to various tests (Figure 6).

Table 4: Rf values of Thin layer chromatography of RCR and RCL.

Plant Sample	Solvent Systems	
	S1	S2
Rumex crispus root extract (RCR)	0.78	0.9
Rumex crispus leaf extract (RCL)	0.8	0.87

TLC-bioautography of prepared sprayed plates of antioxidants and amino acids was carried out against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*. TLC-bioautography of antioxidants and amino acid showed the zones of inhibition around spots on TLC. Zones were clearly seen in Figure 7 around spots as mentioned through arrows. Greater zones were observed in case of RCL as compared to RCR. MTT assay was carried out to check the cell proliferation. Significant reduction of *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa,*

Escherichia coli and *Proteus mirabilis* was found in case of leaf extract of *Rumex crispus* when compared to root extract of *Rumex crispus* (Figure 7).

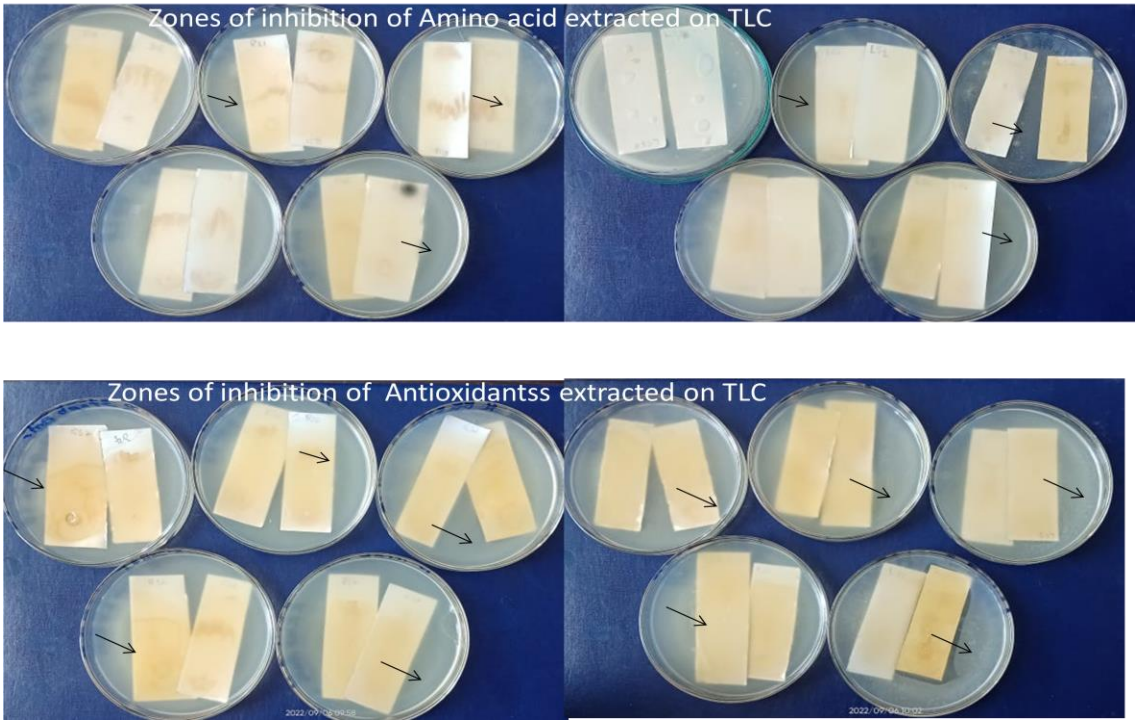
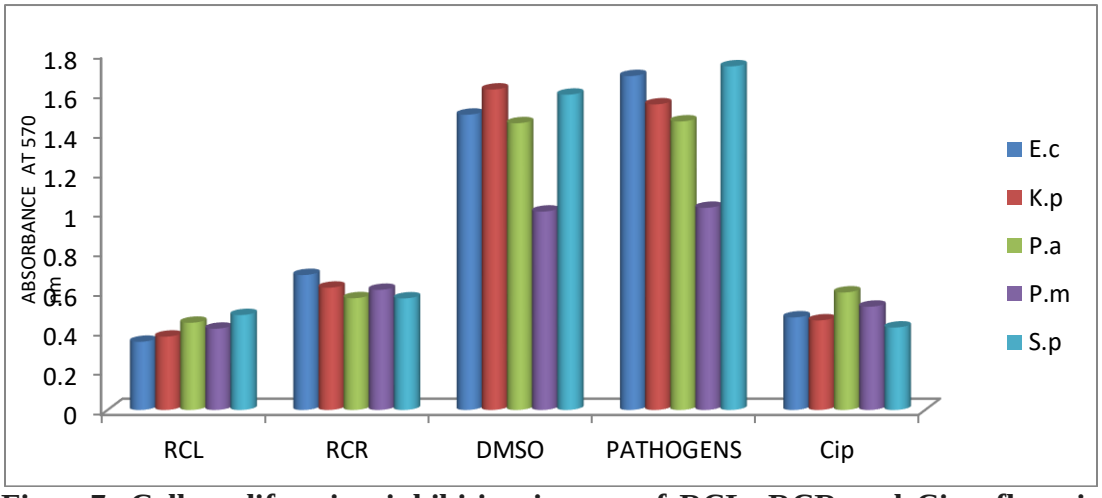


Figure 6: TLC-Bioautography of Amino acids and Antioxidants extracted from *Rumex crispus* separated on TLC plates against *Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.*



Figur 7: Cell proliferation inhibition impact of RCL, RCR and Ciprofloxacin against *Escherichia coli* (E.c), *Klebsiella pneumonia* (K.p), *Pseudomonas aeruginosa* (P.a), *Proteus mirabilis* (P.m) and *Streptococcus pyogenes* (S.p).

DISCUSSION

Traditional healers have employed medicinal plants efficiently in traditional healthcare systems. A prior study looked at the nutritional makeup, minerals, vitamins, anti-nutrients, and essential oils of the medicinal plant *R. crispus'* root and leaf. Except for carbohydrates, which are higher in the root, the leaf contains more ash, crude oil, fiber, and minerals than the root. Phytate was discovered in both the leaf and root of *R. crispus*. The dried leaf had the most retinol, ascorbic acid, and alpha-tocopherol. According to this study, *R. crispus* can be utilized not only for medical purposes, but also as part of a healthy diet (Idris *et al.*, 2019). The current investigation discovered that R. crispus's leaf and root contain a variety of phytoconstituents. When compared to the root, the leaf had more promise in terms of antibacterial activities, synergy with other drugs, and cytotoxicity.

Yildirim *et al.*, 2001, also proved that ether and ethanol extracts of *R. crispus* leaves and seeds showed antibacterial activity in contrast to aqueous extracts. However, they have demonstrated antibacterial activity by disc diffusion method only against bacterial strains *S. aureus, Bacillus subtilis Pseudomonas aeruginosa, E. coli*, and *Candida albicans* .In this current study, the antibacterial activity of the *Rumex* leaves as well as root extracts through agar well diffusion method against *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes* and *Proteus mirabilis Streptococcus pyogenes* were inhibited by all three used concentration (5 mg/ml, 10 mg/ml and 15 mg/ml). Current results showed that leaf extract showed effective inhibitory action as compared to root extract. Antibacterial effect showed that as the concentrations of extracts (RCL and RCR) increased inhibition effect also increased.

Besides the better activity of extracts than single compounds of binary combinations and potential additive/synergistic interactions of the components, the extracts have other beneficial properties, such as antioxidant (Idris *et al.*, 2017) and anti-inflammatory (Singh and Purohit, 2018). Current research showed that aqueous extract of *R. crispus* (RCL and RCR) had effective synergistitic and additive effect when used in combination with antibiotics (Amikacin and ciprofloxacin) via disc diffusion method. This study revealed that sensitivity effect of RCL when used in combination with antibiotics was much more effective than RCR (combined effect with antibiotics). As the concentration increased the inhibition potential in case sensitivity testing of extracts also increased.

The synergy between isolated phytochemicals from different plants against meticillin-resistant *Staphylococci* and *Aeromonas salmonicida* has been previously proven (Coopoosamy and Magwa, 2006; Chukwujekwu *et al.*, 2006) and showed potential effect. There is a lack of data in the literature on the other combinations tested in this study combined effect with antibiotics was more effective then single source.

The antibacterial compounds of the most potent extracts were determined by previously by TLC- bioautography (Dehghan *et al.*, 2020).TLC-bioautography showed that separated phytoconstituents showed inhibitory action against all tested bacteria due to these potent bioactive molecules. This work showed the effective cytotoxic effect to the bacterial pathogens through MTT assay in case of RCL as compared to RCR.

CONCLUSION

This study demonstrated that aqueous *Rumex crispus* leaves extract showed more potential inhibitory action and cytotoxic effect against bacterial pathogens (*Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes* and *Proteus mirabilis*). RCL was more effective when used in combination with standard antibiotics as compared to RCR.

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