ANTIBACTERIAL EFFECT OF *BIDENS BIPINNATA* L. EXTRACT AGAINST SELECTED BACTERIAL STRAINS

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ABSTRACT

The current study was aimed to determine the antibacterial potential of *Bidens bipinnata* L. against bacterial strains isolated from cattle waste. The isolated bacteria included *Bovine brucellosis, Bovine abortus, Bacillus anthracis, Campylobacter coli, Mycoplasma bovis, Campylobacter jejuni* and *Mycoplasma synoviae. Mycoplasma synoviae* represented high resistance to Cephalosporins (Cefoxitin 100%, Cefuroxime 100%, Cefoperazone 100%). *Campylobacter jejuni* and *Bovine brucellosis* showed 100 % resistant to Ampicillin and Cefoxitin, 90 % to Cefuroxime and Cefoperazone, while there was 75 % resistance to Cefotetan. *Campylobacter jejuni* and *Bovine brucellosis* were 65 and 70 % resistannt to Erythromycin. The MAR index for *Campylobacter jejuni* and *Bovine brucellosis* was 0.55 while for *Mycoplasma synoviae*, it was 0.44. Different concentrations of the aqueous extract of the plant were implanted against the resistant bacteria and there was potent antibacterial activity of the plant extract against tested bacteria. It can be concluded that *Bidens bipinnata* has the potential to inhibit/kill the multi-drug resistant bacteria. Active components of this plant may be isolated and should be used against various bacteria to further confirm the antibacterial potency of the plant.

Key words: Bacterial strain, Cattle waste, Multi-drug resistant bacteria, drugs.

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INTRODUCTION

In Pakistan, one of the major sources of proteins is cattle and livestock forming has been started since 1960s. This industry grabbed the attention of the government in the form of relaxation in sale and income taxes and import duties were exempted for a period. All these policies led to an increase in cattle meat consumption up to 4 % each year (Sadiq 2004). In the meanwhile, this industry was also faced several challenges such as diseases in the farms and price variations (Hussain et al. 2015). The high amount of waste produced by cattle industry is also a major challenge which may contribute a high pollutant to environment if not properly disposed (Bolan et al. 2010). The cattle waste contains faecal materials and feed stuffs, and the feathers that could be used for the bedding purposes (Wilkinson et al. 2011).

Many antibiotics have been added to feed supplements as growth promoters (Van den Bogaard and Stobberingh 1999), but massive use of these antibiotics may lead to emergence of multi drug resistance in bacteria (Hajipour et al. 2012). Also, these antibiotics are released into environment and resides in wastes, soil and water (Adelowo et al. 2009). Many of the available antibiotics therefore could not affect the bacterial stain which are resistant (Lynch et al. 2007) and the infections caused by these resistant strains are increasing with regular interval. These resistant strains cause both nosocomial as well as non-nosocomial infections throughout the world (Franci et al. 2015). As the massive use of antibiotics develops resistance in bacteria (Cooper et al. 2002) and these infections are difficult to be treated by the available antibiotics (Taylor et al. 2002), hence, new drugs are to be produced which can counteract the infections caused by these resistant bacteria (Morones et al. 2005).

Plants have been used in medicine since a long time all over the world and is one of the major therapeutic agents used traditionally (Gupta et al. 2007). Plants have significant medicinal importance as its chemical constituents can cure illness, boost up immune system and can prevent diseases (Namukobe et al. 2011). *Bidens bipinnata* belongs to the Asteraceae family, and is widely distributed in tropical, subtropical and temperate regions of the world. *Bidens bipinnata* is widely utilized medicinal plant for treatment of diseases like malaria, sore throat, acute nephritis and dysentery (Yuan et al. 2008). However, despite its traditional uses *Bidens bipinnata* is not widely explored for its antimicrobial effect. Thus, the current study has been designed to investigate the antibacterial potential of *Bidens bipinnata* L. aqueous extract against selected antibiotic resistant bacterial isolates.

Materials and Methods

Sample collection

Samples (bacterial strains) were taken from different parts of farming area where the cattle's were kept for raising. The samples were collected in screwed test tubes and all the samples were transferred to laboratory and different growth media were prepared. The bacterial colonies isolated from the cattle waste were confirmed based on morphological characteristics, staining and biochemical properties (Abiala *et al*., 2016).

Antibiotic sensitivity test and antimicrobial potency of *Bidens bipinnata*

The commercially available antibiotic discs were applied in replicates on the plates and the diameter of zones of inhibition were measured. *Bidens bipinnata* aqueous extract was used for determination of antibacterial potency against the isolated bacterial strain. The isolated bacteria were inoculated on MH agar plate and already prepared discs of various concentrations from the aqueous extract were placed on the surface of the media. The zones of inhibition were recorded after 24 hours of incubation.

Multiple antibiotic resistances

Multiple antibiotic resistivity was determined according to the method described by (Krumperman 1983). For a single isolate, the MAR index was calculated as; total number of antibiotics to which the isolate was resistant divided by total number of antibiotics used against the same isolate.

RESULTS

3.1: Isolation of bacteria and antibiotic sensitivity assay

Several bacteria were isolated from the cattle waste. Among the isolates, most were gram negative bacterial isolates while a low number of gram-positive were also present. The cattle were observed to be the source of isolates included *Bovine brucellosis, Bovine abortus , and Bacillus anthracis* along with *Campylobacter coli, Mycoplasma bovis* and *Campylobacter jejuni*. *Mycoplasma synoviae* was found as the leading gram-positive isolate present in the cattle litter (Table 1).

Table 1: Shows Biochemical and morphological and Characteristics

Bacteria	Gram + or -	Morphology	spores	catalase	Oxidases	lactase	dehydrogenase	Glutamate	H2O2
M. synoviae	+	*	-	+	+	+	-	-	+
B.anthracis	-	*	-	+	+	+	+	-	+
M.bovis	-	*	-	-	+	+	-	-	+
C.r coli	-	*	-	-	-	-	-	-	-
C. jejuni	-	*	-	+	+	+	+	-	+
E. coli	-	*	-	-	-	-		-	-
B.brucellosis	-	*	-	+	+	+	-	-	+

Note: *= Rod shape, - = absent, + = Present

3.2: Bacterial resistance of bacteria to various antibiotics

Bacillus anthracis (*Bacillus anthracis*) and *Bovine abortus* (*E. coli*) showed 55 % and 60 % resistance to Ampicillin, respectively. *Bacillus anthracis* and *E. coli* were 60 % and 80 % resistant to

Cefoxitin, 80 % and 65 % to Cefuroxime and 65 % and 70 % to Cefoperazone, respectively These two bacteria were not resistant to Cefotetan. A 55 % resistance was observed against Erythromycin when both the bacteria were used against it. There was 10 % resistance to Norfloxacin, Ofloxacin and Ciprofloxacin by *Bacillus anthracis* and 15 % resistance by *E. coli* to these antibiotics. Both *Mycoplasma bovis* and *Campylobacter coli* had 100 % resistance to Ampicillin. *Mycoplasma bovis* had 85 % resistance to Cefoxitin, 80 % and 85 % to Cefuroxime and Cefoperazone, and 55 % resistance to Cefotetan, respectively. *Campylobacter coli* had almost the same pattern of resistance to Cephalosporin; 70 % resistance to Cefoxitin, 70 % and 75 % to Cefuroxime and Cefoperazone, and 55 % resistance to Cefotetan, respectively. Both *Mycoplasma bovis* and *Campylobacter coli* had 60 % resistance to Erythromycin. There was 25 % resistance to Norfloxacin, 35 % to Ofloxacin and 20 % resistance to Ciprofloxacin by *Mycoplasma bovis*. Further, *Campylobacter coli* showed 25 % resistance to Norfloxacin and Ofloxacin and 15 % resistance to Ciprofloxacin. *Campylobacter jejuni and Bovine brucellosis* were more resistant to the antibiotics used; 100 % resistance was recorded for Ampicillin, and Cefoxitin, 90 % for Cefuroxime and Cefoperazone, while there was 75 % resistance to Cefotetan. *Campylobacter jejuni* and *Bovine brucellosis* were 65 % and 70 % resistannt to Erythromycin. *Campylobacter jejuni*

and *Bovine brucellosis* were 35 % and 65 % resistance to Norfloxacin, 40 and 20 % to Ofloxacin and 15 and 10 % resistance to Ciprofloxacin, respectively (Table 2, Figure 1).

Table 2: Antibiotic susceptibly of isolated bacteria against various antibiotics

Bacteria	Antibacterial agent (Percent resistance)								
	AMP (%)	**CEF (%)**	**CEFU (%)**	**CEFO (%)**	**CEFOT (%)**	**ERY (%)**	**NOR (%)**	**OFL (%)**	**CIP (%)**
M. synoviae	25	100	100	100	35	75	20	20	20
Bacillus anthracis	55	60	80	65	00	55	10	10	10
Mycoplasma bovis	100	85	80	85	55	60	25	35	20
Campylobacter coli	100	70	70	75	55	60	25	25	15
C. jejuni	100	100	90	90	75	65	35	40	15
E. coli	60	80	65	70	00	55	15	15	15
P.aeruginosa	100	100	90	90	75	70	65	20	10

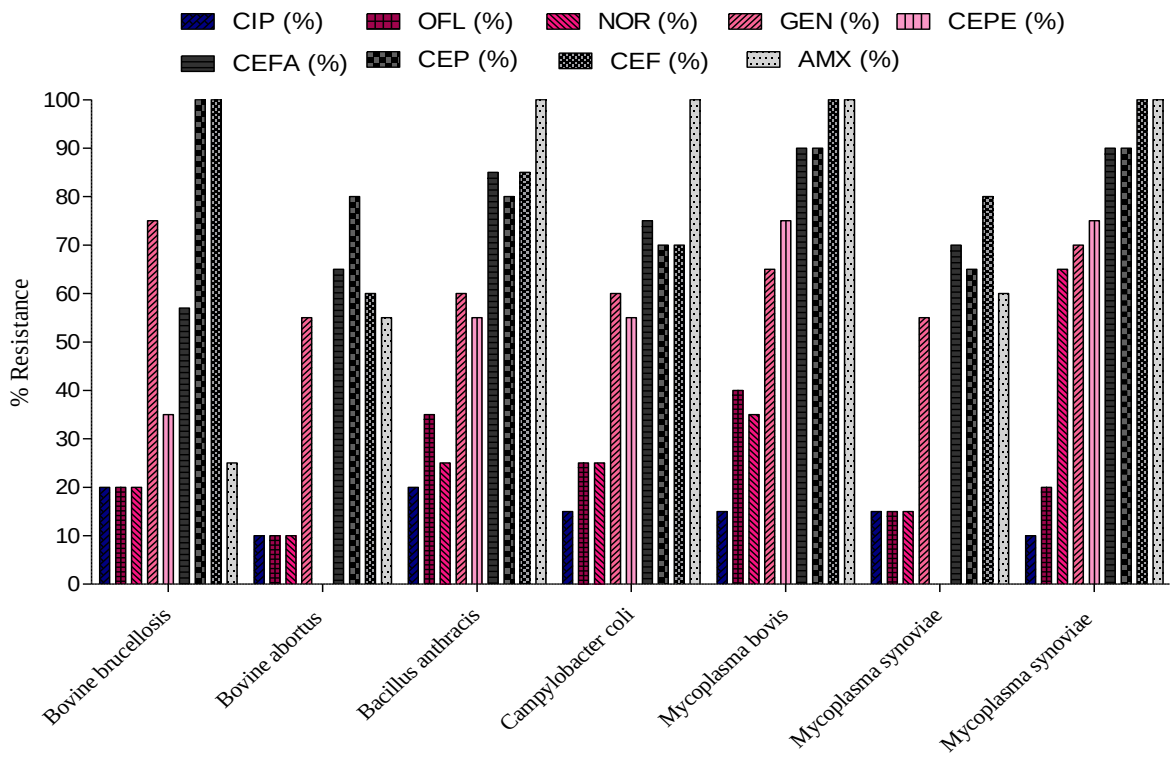

Figure 1: Graph showing the susceptibility pattern of different antibiotics against various bacteria

3.3. Multiple antibiotic resistance indexes have been given in table no 3 for each bacterium.

Table 3: Multiple antibacterial resistance index of different bacteria

Bacteria	No of antibiotic to which the bacteria are resistant	Total no of antibiotics used against the bacteria	MAR index
M. synoviae	4	9	0.44
Bacillus anthracis	1	9	0.11
Mycoplasma bovis	4	9	0.44
Campylobacter coli	2	9	0.22
C. jejuni	5	9	0.55
E. coli	1	9	0.11
P.aeruginosa	5	9	0.55

3.4. Antibacterial action of Bidens bipinnata L.

Different concentrations of the aqueous extract of the plant were made, discs were prepared and these extracts with different concentrations. Results obtained have been given in Figure .

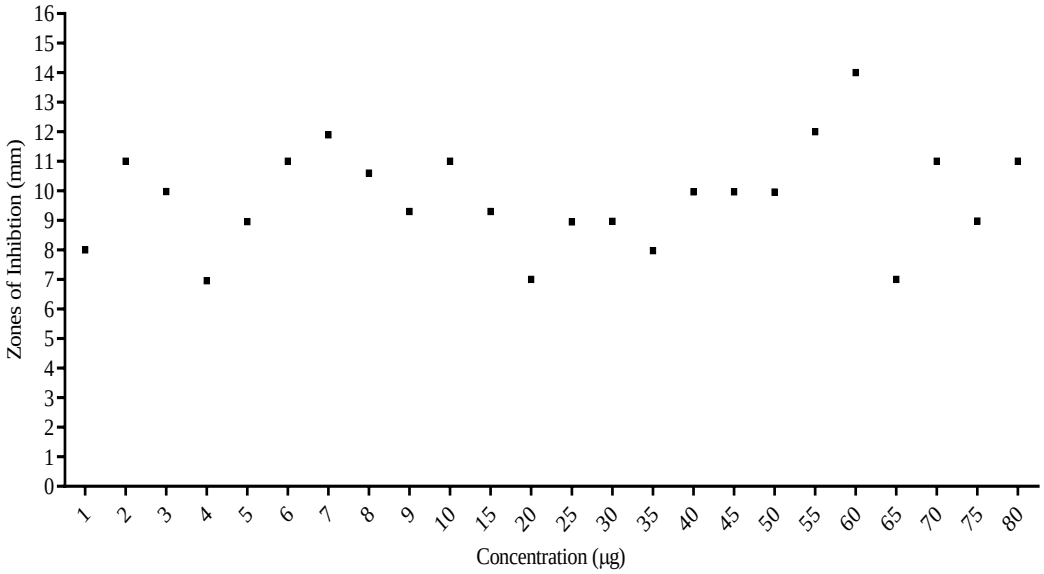

Figure 2: Graphical representation of antibacterial potential of *Bidens bipinnata* L. against *Mycoplasma synoviae*

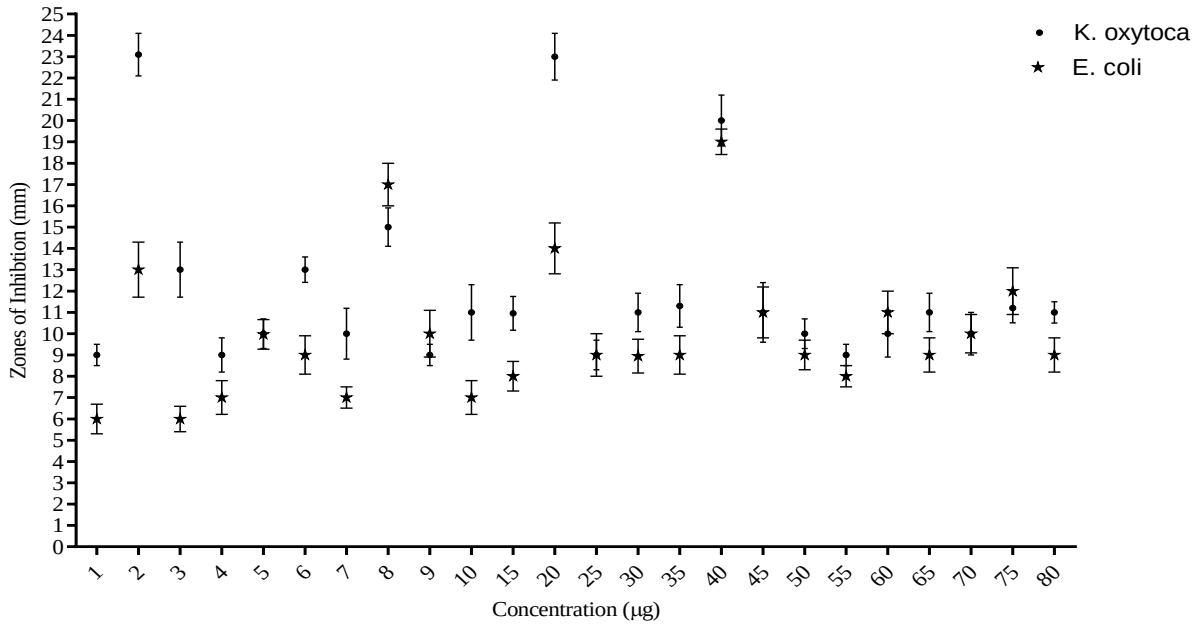

Figure 3: Graphical representation of antibacterial potential of *Bidens bipinnata* L. against *Bacillus anthracis and Bovine abortus*

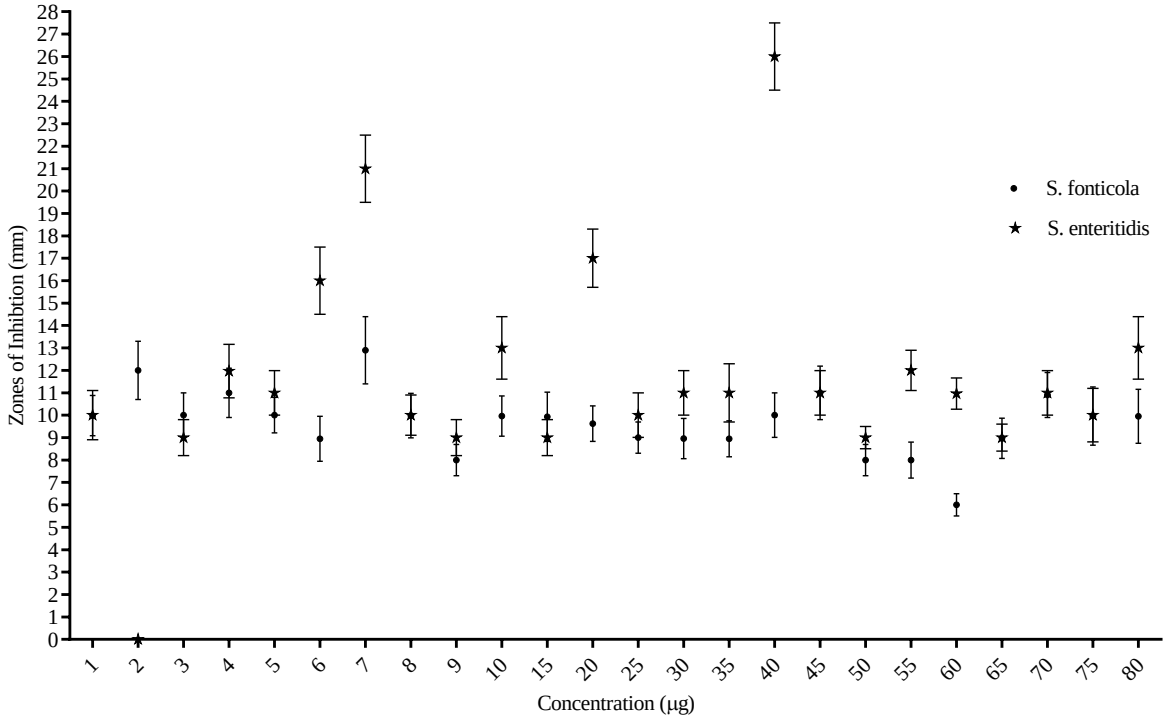

Figure 4: Graphical representation of antibacterial potential of *Bidens bipinnata* L. against *Mycoplasma bovis* and *Campylobacter coli*

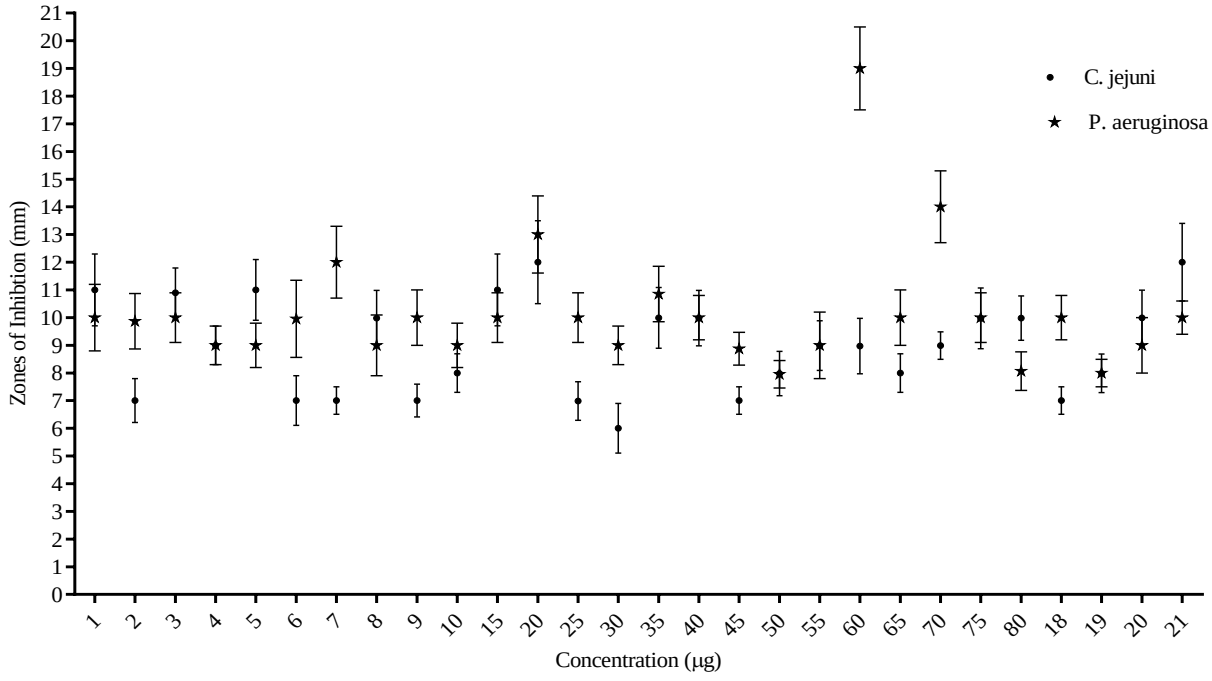

Figure 5: Graphical representation of antibacterial potential of *Bidens bipinnata* against *Campylobacter jejuni* and *Bovine brucellosis*

DISCUSSION

Cattle waste is composed of the excreta along with the hairs, and dusts. Most of the cattle industry produces milk, meat and their litter contains wood chips, sawdust, wheat straw, and rice hulls (Wiliams et al. 1999). Similarly, it also contains plant crude protein, minerals and few heavy metals (Martin et al. 1998). Hence, cattle litter acts as manure but it also contaminates the environment due to the presence of nitrate, and phosphate that enters the water and also gives purulent gases i.e. ammonia to air (S'MITH 1974, Stüven and Bock 2001). Furthermore, this waste also could be a growth promoter for the cattle (S'MITH 1974). Besides, there could be a lot of pathogenic microorganisms that causes infections in cattle and in human when come in contact via contaminated soil and water (Martin et al. 1998).

Cattle are mostly grown in the same litter in the farms without removing the older litter (Vizzier Thaxton et al. 2003) and this practices increases the risk of diseases in the new flock (Macklin et al. 2008). Decomposition can decrease all these risks to control viruses (Giambrone et al. 2008) and bacteria (Macklin et al. 2006).

In present study, many bacteria were isolated from cattle litter; one was gram positive and the rest were gram negative. Few of them were gas producing bacteria, some were motile, few of them were glucose, lactose and sucrose fermenter. Spore producing bacteria were not detected. Among those bacteria, *M. synoviae, Bacillus anthracis, Mycoplasma bovis, Campylobacter coli, C. jejuni, E. coli* and *Bovine brucellosis* were identified. *C. jejuni* can contaminate the farm lands and hence affects the fodder and contaminate environment, promotes food borne diseases (Alsarhan *et al.*, 2014). Amran, (2009) have reported that *M. synoviae* produces bacteriocin which can reduce and control other bacterial infections. Beg and Ahmad, (2000) have documented the presence of *Mycoplasma bovis* and *E. coli* in cattle waste.

In the present findings, Quinolones were highly effective against the tested bacteria. A high percentage of susceptibility was found for Quinolones (Norfloxacin, Ofloxacin and Ciprofloxacin). The Erythromycin and Cefotetan had almost the same level of susceptibility against the tested bacteria. Ampicillin showed least susceptibility. Similarly, *Campylobacter jejuni* was 100 % resistant to ampicillin and 90 % to Cefoxitin, Cefuroxime and Cefoperazone, while 75 % to Cefotetan. These results are in deviation from the results of (Ogbor et al. 2019) as there was 100% resistance to nalidixic acid, chloramphenicol, cloxacillin, and streptomycin, 87.5% were susceptible to ampicillin. The reason may be the use of different species of Campylobacter in the present study as in no specie was identified.

C. jejuni showed 100 % resistance to Ampicillin in present study, same findings have been reported where amoxicillin-resistant commensal Campylobacter have been documented as Amoxicillin therapy alone have no effect and the Co-amoxiclav had high efficacy against the amoxicillin resistant bacteria (Elviss et al. 2009). It has been suggested that as there is emergence of high resistance to many antibiotics, there is the need of careful use of antibiotics in cattle industry (Gharbi et al. 2018). The *Klebsiella* specie showed resistance to ampicillin and were susceptible to Cephalosporins, aminoglycosides, and quinolones (Stock and Wiedemann 2001).

Drug resistance is increasing day by day throughout the world and there is need to identify new chemicals that have the potency against the resistant bacteria. In present study, *Bidens bipinnata* L. showed high efficacy against the tested bacteria. There is high potency of this plant against the bacteria that were isolated from the cattle waste in the farms. It can prevent cattle infections caused by test organisms. Petroleum ether, chloroform, benzene, methanol, ethanol and water extracts of *Bidens pilosa* L. was used to check the bactericidal potency and it has been found that organic solvents extract the active components in high amount than the aqueous, which are active against the bacteria (Silva *et al*., 2011). This plant is used as food in many countries like Russia, and China, as it contains high amount of vitamins and coumarins, isoflavones, naphthoquinones, alkaloids and saponins (Sadowska et al. 2014). The leaves of *Bidens bipinnata* were boiled in water, cooked, and used to treat the constipation and indigestion (Abbasi et al. 2013). Medicinal plants play a major role in this regard.

CONCLUSION

It could be concluded that *Bidens bipinnata* has potential antibacterial properties against the various selected resistant bacterial strains; therefore, it should be very good to investigate the plant following recent techniques i.e isolate the active ingredients of this plant will help in the treatment of many infections caused by various bacteria and other pathogens. Livestock diseases cost farmers millions of pounds a year. Farmers must also know that many of the diseases that live in stocks go from animal to animal to human. Animals usually acquire diseases either by contact with diseased animals or improper sanitation, nutrition, care, and management. Prevention of diseases is better and more economical than rushing to control disease outbreaks.

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