Original Research Article

In Vitro Antimicrobial Activity and Cytotoxicity Test of Native South Dakota Plant Extracts

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A B S T R A C T

Keywords

Antimicrobial activity,
Escherichia coli, Methanol extract,
Minimum Inhibitory Concentration,
Cytotoxicity

In vitro antimicrobial activity of 28 extracts of plants native to South Dakota were screened and evaluated against *Escherichia coli*. A disk diffusion assay was used for initial screening and antimicrobial activities of potential plant extracts were further assessed by a broth dilution method to determine their MIC values. Out of 28 plant extracts five plant extracts from *Clematis ligusticifolia, Monarda fistulosa, Rhus aromatica, Centaurea stoebe* and *Onosmodium molle* showed significant antimicrobial activity. In disk diffusion assay the percentage of inhibition was maximum for *C. ligusticifolia*. The minimum inhibitory concentration (MIC) for *C. ligustifolia* was 0.031gm /ml, for *C. stoebe* was 0.062gm /ml, for *O. molle* and *M. fistulosa* it was 0.125gm /ml and for *R. aromatica* 0.25gm /ml. In vitro cytotoxicity tests indicated that *C. ligustifolia* and *R. aromatic* showed some toxicity to porcine intestinal epithelial jejunum-2 cells (IPEC-J2) whereas *C. stoebe* did not have impact on cell growth compared to controls. However *O. molle* and *M. fistulosa* increased the growth of IPEC-J2 cell. Based on our findings it can be concluded that these five plants have antimicrobial activity against *E. coli*. Further investigation to determine which of their secondary metabolites (phytochemicals) are biologically active will be pursued.

Introduction

Around the middle of 20th century, major advances in antibacterial drug development and other means of infection control helped human beings triumph over many infectious diseases. Development of penicillin in the early 1940s changed the situation radically with respect to bacterial infection (Tenover, 2006). However, the attainment of complete eradication of infectious diseases is almost impossible. Bacteria respond to antibiotics by developing various modes of resistance as fast as we use antibiotics. Antimicrobial resistance in bacteria is one of the biggest global concerns at present (Siddiqi *et al.,* 2011). Multidrug resistant isolates in several infectious agents are a cause for serious

concerns. *Escherichia coli* isolates from environmental, animal and human sources have been identified as resistant to antibiotics (Heike and Reinhard, 2005). This in part is the consequence of improper use of antibiotics as medicine. Pathogenic organisms have acquired some degree of resistance toward antimicrobials through various mechanisms (Wose Kinge *et al.,* 2010). Three types of antibacterial resistance strategies have been suggested. A drug efflux pump has been observed in several bacteria which prevent accumulation of antibiotics. A second mechanism is deactivation or destruction of the antibiotics by hydrolytic enzymes in the periplasmic space and a third strategy for antibiotic resistance appears to be reprogramming of the target macromolecules to reduce the affinity of antibiotics for their RNA (Walsh, 2000; Tenover, 2006; Alviano and Alviano, 2009). Resistance has led to need for development of alternate sources of antibiotics to control *E. coli* infections.

Ancient herbal traditions are the source of origin for numerous contemporary medicines (Al-Bakri and Afifi, 2007). For the past few decades researchers have been trying to identify novel antibiotics from plant products. Medicinal plants used in folk medicine may provide guidance for developing new antibiotics. Higher plants produce large numbers of secondary metabolites with different biological activities. Medicinal plants have been used for centuries to treat various diseases all over the world (Vaghasiya and Chanda, 2007). So far, only a small percent of traditionally prescribed plant species on the earth have been studied for their therapeutic value. Plant phytochemicals could provide alternative classes of antibiotics having different target sites than current antibiotics, which may be effective against drug resistant pathogens. The drug industry is

exploring phytochemicals extensively to develop new antibiotics which can overcome resistant pathogens without any toxic effect to their host (Oskay and Sarı, 2007). However, recent events show that drug companies are abandoning antibiotic research as Pfizer exit for the market suggests the need for academic research where knowledge - not profit are still important. Plants contain a large number of secondary compounds like alkaloids, phenolic compounds and flavonoids having antimicrobial properties.

There are several reports presented on the antibacterial activity of organic and aqueous plant extracts. *Casearia sylvestris* is a popular medicinal plant of South America and is being used against several diseases; diarrhea, gastric ulcer, inflammation, and herpes. Furthermore it was found that the ethanol extracts from leaves of this plant have potential antimicrobial activity (da Silva *et al.,* 2008). In a recent study methanol extracts of *Globularia alypum* have shown significant effects on the growth of *E. coli* (Bogdadi *et al.,* 2007). *Berberis asiatia,* a plant thatis traditionally used in Nepal and India to treat wounds, contains alkaloids that show strong activity against gram negative bacteria (Bhandari *et al.,* 2000). Antimicrobial activity of native plants of Jordan; *Gundelia tournefortii* L. and *Piminella anisum* L. have shown demonstrated activity against antibiotic resistant strains of *E. coli* whereas, *Origanum syriacum* L, *Eruca sativa* Mill extracts have synergistic effects when used with the antibiotic clarithromycin (Darwish and Aburjai, 2010). Leaf extracts of three different species of Aloe; *Aloe barbadensis*, *A. chabaudii* and *A. arborescens*, used in folklore veterinary medicine in Zimbabwe were evaluated for their antimicrobial activity and the study showed that *E. coli* are sensitive to all three extracts (Mbanga *et al.,*

2010). Dubey *et al.* (2009) screened medicinal plants of India for their antimicrobial activity against *E. coli* and found four plants *Terminalia catappa*, *Syzygium cumini*, *Eucalyptus hybrid* and *Holarrhena antidysentrica* have significant antimicrobial activities.

Native Americans have used herbs medicinally for thousands of years as a part of their holistic approach to good health (Moerman, 1998). Anthony (2001) in his book has described a number of herbal remedies used by Native Americans to treat illness and heal injuries. Borchardt *et al.* (2008) found seven plant species that showed some effect against *E. coli* from Minnesota and Wisconsin. In South Dakota there are many varieties of plants that have been used as folk medicine, only few of them have been studied scientifically for their antimicrobial properties. Our main objective in this study was to identify South Dakota medicinal plants having significant antimicrobial activity that could be exploited as a source of antimicrobial agents. We also evaluated their potential for treating bacterial infection without significant toxicity to mammalian cells. In this article we have identified five medicinal plants having antimicrobial activity against *E. coli*.

Material and Methods

Selection of plants: Plants were selected (Table 1) based on their traditional use by Native Americans (Moerman, 2009).

Sample collection: Plant specimens for our study were collected from the Northern Great Plain from June through August 2010. Plants were collected from different locations weighed and stored at -80°C if not used immediately. Identification of the plants species was made based upon the USDA plant database nomenclature.

Voucher specimens were prepared and stored in the herbarium.

Extract preparation: Plant samples were homogenized in a blender with methanol at a ratio of 25gm fresh weight/ 250ml methanol. Plant materials were extracted with methanol for about 24 hours in dark. The plant samples were then vacuum filtered using VWR grade 415 qualitative filter paper. Methanol was removed from the sample using a rotary evaporator under vacuum. The residue was solubilized in 5 ml of 70% ethanol with concentration representing 5 g fresh plant weight per ml of ethanol in the final plant extract.

Bacterial strains and growth conditions

Escherichia coli ATCC # 25922 were obtained from the South Dakota Veterinary Diagnostic Laboratory at SDSU. The assay medium for *E. coli* was Trypton Soy Broth (TSB) or Agar (TSB, TSA, Oxoid Ltd. Hamsphire, UK). Bacterial cultures for antimicrobial testing were prepared by inoculating 25ml of TSB from fresh culture plates. Cultures were grown overnight in a shaker incubator at 200rpm and 37°C (Klancnik *et al.* 2010). For antibacterial activity assay the CFU is adjusted to 10^6-10^7/ml. Stock culture were maintained in 70% glycerol and stored at -80°C.

Antimicrobial testing methods

Disk Diffusion Assay (DDA): The disk diffusion assay was performed following the protocols of National Committee for Clinical Laboratory Standards (NCCLS, 2003) a modified Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). One hundred µl of bacterial culture was evenly spread on a Mueller Hinton agar medium in a petri dish. Sterile6mm diameter paper disks were impregnated with 20µlplant extract. The

disks were allowed to completely air dry. Disks were then placed on the inoculated agar plates. Each extract was screened with three replicates. One disk of gentamicin (10µg per disk) was placed on each plate as positive control and one disk of ethanol dried as above, as negative control. The inoculated petri dishes were incubated for 18-24 hours at 37°C. After incubation the diameter of the inhibition zones were measured in mm.

Broth Microdilution method for Minimum Inhibitory Concentration (MIC): The MIC value of the plant extracts that were active against *E. coli* in the disk diffusion assay was then measured. For the MIC value the method described by OrdoñEz *et al.,* 2003 and Sherlock Orla *et al.,* 2010 were followed with some modifications.

Using 96- well plate (Corning, NY), columns 1 and 2 were filled with 50 µl sterile water and column 3 with 50 µl of 70% ethanol. In the remaining columns, the first row was filled with 90 µl of sterile water. The remaining wells were filled with 50 µl of sterile water. Then, 10 µl of each extract was mixed with the 90 µl of water in the first row. To obtain a twofold serial dilution of each extract ranging from 0.5 g/ml to 0.0039 g/ml of plant extract in fresh weight, 50 µl of the first row were aspirated with a micropipette and mixed with the second row. This process was repeated till the last row with remaining 50 µl was discarded resulting in all the wells containing a total of 50 µl of diluted extract.

Then, 50 µl of TSB with bacteria (10^6 CFU/ml) was added to all the wells except those in the first column, which served as blanks. Fifty µl of TSB without bacteria was added to column one. Column 2 was negative control and column 3 was positive

control. Finally the 96 well plate was incubated at 37°C for 18 hours. The bacterial growth was observed by taking absorbance at 600 nm. All assays were performed in triplicate, with absorbance of extract only and blanks subtracted to adjust for background absorbance.

The MIC value was termed as the minimum concentration of plant extract that inhibited growth of *E. coli* to a level < 0.05 at 600nm.Growth at this level cannot be observed through microscope (OrdoñEz *et al.,* 2003).

The minimum bactericidal concentration was also determined by using the plate streaking method. In this method a loopful of the content of the well was streaked onto a sterile tryptic soy agar plate and allowed to incubate for an additional 24 hours, if there was bacterial growth within 24 hours it was concluded that extract acted as a bacteriostatic. If after 24 hour there no visual sign of growth the extract was determined to be bactericidal.

Cytotoxicity test

DNA – based proliferation assay: The DNA based proliferation assay was performed as per the instruction provided by Roche Molecular Biochemicals (Catalog Number: 11669915001) briefly, undifferentiated 7500 porcine intestinal epithelial cells (IPEC-J2) that were derived from one day old pig jejunum (Koh *et al.,* 2008) were cultured in 100 µl media in a 96 well flat bottom plate and incubated for 24 hours at 37ºC. Ten µl of media was replaced with 10µl of plant extract with a final volume 100µl in each well. Cells were incubated for 18- 24 hours at 37°C. All cells in 96 well plate were labeled with BrdU labeling solution (10µl per well). The plate was incubated for 18 hours at 37° C, the

media was aspirated, and 200µl of FixDenat solution was added to each well and incubated for 30 min at room temperature. FixDenat solution was removed and 100ul anti-BrdU-POD working solution was added and incubated for 90 min at room temp, followed by washing and the addition of substrate solution. Plates were incubated for 20 min and quenched with H_2SO_4. Absorbance was measured at 450 and 690 nm (back ground correction).

Result and Discussion

Twenty eight plants species were collected for our study (Table 1). The antimicrobial activity of these plant extracts were screened by disk diffusion assay. Out of twenty eight plants five plants showed significant antimicrobial effect on *E. coli. Centaurea stoeba*, *Rhus aromatica*, *Monarda fistulosa* and *Onosmodium molle* showed moderate inhibition to *E. coli* growth whereas *Clematis ligusticifolia* showed maximum inhibition (Fig. 1). *C. ligusticifolia* was found to be highly effective against *E. coli* having zone of inhibition of 24.75 mm which was even greater than gentamicin (Fig. 1). *R. aromatic* and *C. stoebe* showed zone of inhibition that was more than 50% as large as that caused by gentamicin. *M. fistulosa* and *O. molle* showed inhibition distances less than 50% of that of gentamicin.

The disk diffusion assay provides an initial screening method showing that extracts from these 5 plant species have the potential for use as antimicrobials. To further assess these plants we determine their MIC value by broth dilution method and evaluate whether the effect of these extracts were bactericidal or bacteriostatic.

Broth Microdilution Method

The minimum inhibitory concentration

(MIC) is the lowest concentration at which an antimicrobial substance inhibits microbial growth under specified conditions. This concentration is bacteriostatic as it inhibits the growth but does not kill the bacteria completely. *C. ligusticifolia* showed the lowest MIC value, 0.031 g/ml whereas the highest MIC value was for *R. aromatica* 0.25 g/ml (Table 2).

The minimum bactericidal concentration (MBC) is defined as the lowest concentration at which the test compounds kill the bacteria. The MBC values for all five plants were found to be 0.5gm/ml (Table 2).

Cytotoxicity Test

To determine the toxic effect of these plant extract on animals we performed cytotoxicity test using DNA- based proliferation assay. The result from this assay showed a wide range of effect of the plant extracts on the animal cell (IPEC-J2) (Fig. 2). *Clematis ligusticifolia* and *Rhus aromatica* both showed cytotoxicity to the IPECJ2 cells. A total of eight different concentrations had been examined from 0.0039 gm/ml to 0.5gm fresh weight /ml. *C. ligusticifolia* did not show any change in its toxic effect even at the lowest concentration whereas *Rhus aromatica* decreased in cytotoxicity as the concentration of the extract decreased (Fig. 3 and 4).

The extract of *C. stoebe* did not alter the growth of IPEC-J2 cells, when compared to the control. The extracts of *M. fistulosa*and *O. molle* appeared to increase the growth of IPEC-J2 cells at their higher concentration. These extracts significantly increased incorporation of BrdU by greater than two fold compare to control (Fig. 2). *R. aromatica* and *C. ligusticifolia* reduce the cell growth below 50% compare to control.

All plants tested have been used traditionally by the American Indians. Many of these species were utilized alone or in combination with other plants to treat a wide range of ailments. Only five plants out of 28 were effective against *E. coli*. The results of MIC assay showed that some of these plant extracts may provide potential sources of new antimicrobials. The BrdU assay indicated that only *C. ligusticifolia* was the most toxic to cultured IPEC-J2 cells *R. aromatica* also showed some toxicity against IPEC-J2 cells. Tetracyclines, which are broad spectrum antibiotics and widely used for various bacterial infections, have also been shown to induce cytotoxic effects in human blood lymphocytes *in vitro* (Celikand Eke, 2011). Erythromycin and its chemical derivatives are used to treat variety of human infections. These antibiotics have also been reported to cause cytotoxicity in human liver cell lines when treated *in vitro* (Viluksela *et al.,* 1996). Additionally antibiotics ciprafoxacin, clyndamicin and metronidazole were tested on human gingival fibroblast cell cultures and showed cytotoxicity effect in a dose dependent manner (Ferreira *et al.,* 2010). Though these antibiotics showed toxicity *in vitro* test, still they are in use to treat bacterial infections. To confirm the safety use of *C. ligusticifolia* and *R. aromatica* further *in vivo* studies are necessary.

The genus *Clematis* (Family – *Ranunculaceae*) includes 350 species which grow worldwide (Dong *et al.,* 2010). Pharmacological studies have shown that extracts of many species of this genus demonstrate antimicrobial activity (Buzzini and Pieroni, 2003). Khan *et al.* (2001) reported that *Clematis papuasica* leaves and stem bark have broad spectrum antimicrobial activity. Buzzini and Pieroni (2003) studied antimicrobial activity of *Clematis vitalba* and Kyung *et al.* (2007) reported the antimicrobial activity of

Clematis apfifolia DC. Bioactive components of different species were identified and isolated by researchers. Phytochemical researches carried out on *Clematis mandshurica* revealed the presence of triterpenoidsaponins (Dong *et al.,* 2010), lignans, alkaloids (Shi *et al.,* 2006b), macrocyclic glycoside (Shi *et al.,* 2007) and phenolic glycosides (Shi *et al.,* 2006a). Wu *et al.* (2010) studied the therapeutic action of *Clematis chinensis*. However the nature and chemical constituents of *Clematis ligusticifolia* have not been extensively studied. The Native American used an infusion of leaves on horses for wounds, treatment of skin disease, ulcers and colds (Sweet.M, 1998). *C. ligusticifoila* extract inhibited *E. coli* even more than gentamicin (positive control). It also inhibited cell growth in the IPEC J2 culture across a range of serial dilutions. Although this extract was highly effective against *E. coli*, additional research needs to be performed to determine how these extracts can be used as antibiotics and which of the compounds in the extracts are responsible for their activity.

Antimicrobial effect of *M. fistulosa* (Family-*Lamiaceae*) has been studied by various researchers. Zhilyakova *et al.* (2009) studied the essential oils of *M. fistulosa* and showed that they have antibacterial activity against Gram negative bacteria. GC-MS analysis of Monarda oil revealed that thymoquinone (simple monoterpene quinine) is the major constituent and that it shows antimicrobial activity against *Escherichia coli* (Inouye *et al.,* 2006). Johnson et al (1998) reported the isolation of two bioactive monterpenesthymoquinone and thymol and also observed that the reduction of thymoquinone to thymohydroquinone reduced its activity. Our results showed that it is toxic to *E. coli* but not toxic to the animal cell line and therefore it may be used as a potential antibiotic to control *E. coli* infections.

The genus *Centaurea* (Family – *Asteraceae*) includes over 500 species that are found all over the world (Tekeli *et al.,* 2011).Various species of *Centaurea* have been used traditionally to treat diseases such as hemmorhoids, abscesses and common colds. Twelve *Centaurea* species has been studied in Turkey for their antimicrobial activity. Eight of these species (*C. balsamita*, *C. calolepis*, *C.cariensis* subsp. *maculiceps*, *C. cariensis* subsp. *microlepis*, *C. kotschyi* var. *kotschyi*, *C. solsitialis* subsp.*solsitialis*, *C. urvillei* subsp. *urvillei* and *C. virgata*) demonstrated significant antimicrobial activity against different microorganisms (Tekeli *et al.,* 2011). Guven *et al.* (2005) reported that ethyl acetate extracts of *C.odyssei* and *C. kurdica* have significant antimicrobial effect. The chemical composition of *C.austro-anatolica* has been determined by GC-MS and the major components they found were caryophyllene oxide, spthulenol, n-tricosanol and geranyl isovalerate(Ugur *et al.,* 2009). The main classes of components are oxygenated monterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpines and aromatic alcohols (Ugur *et al.,* 2009). The key compound of essential oil in *C. sessilis* and *C. armena* was found to be β-eudesmol and was shown to have antimicrobial activity against both gram positive and gram negative bacteria (Yayli *et al.,* 2005). Many species of *Centaurea* have been found to be effective against a broad spectrum of microorganism, however there is little published research concerning the chemical constituents of *Centaurea stoebe*. Further investigation to isolate the bioactive compounds of *C. stoebe* will be necessary.

Genus *Rhus* contains 250 species in the family of Anacardiacea found in temperate and tropical region worldwide. Various species have been used by the native people for medicine (Rayne and Mazza, 2007). Gundidza *et al.* (2008) reported the presence of α-pinene (86.95%) in the essential oil of *Rhus lancea* having antimicrobial activity against *E. coli*. *Rhus coriaria* is used as a spice in Turkey and has been shown to have antimicrobial activity against *E. coli* 0157: H7 (food borne pathogen) (Nasar-Abbas and Halkman, 2004). Shabana *et al.* (2008) reported that the major constituents of essential oil of *Rhus coraria* fruits are thymol, caryophyllene and embrene which are the key components with antimicrobial activity.

It is also reported that *Rhus coraria* had significant antimicrobial activity against several gram positive and gram negative bacteria including *E. coli* (Fazeli *et al.,* 2007). Mossa et al (1996) described the presence of free flavonoids such as persicogenin, velutin, (2S) 5,3',4'-trihydroxy 7-methoxyflavonone and homoeriodictyol in *Rhus retinorrhoea*. Leaves, stems, barks, roots, fruits and the galls on *R. chinensis* have been shown to have therapeutic value for treating diarrhea, dysentery, rectal and intestinal cancer, diabetes mellitus, sepsis, oral disease and inflammation (Djakpo and Yao, 2010). Very little information is available about the phytochemical constituents and antimicrobial activity of *Rhus aromatica* the fragrant sumac which is native to the Northern Great Plains and used by the Native American for various medicinal purposes. Reichling *et al.* (2009) have reported that the aqueous extract of *R. aromatica* has antiviral potency against herpes simplex virus type 1 and type 2. In our research we demonstrated the antimicrobial activity of *Rhus aromatica* against *E. coli*. Further research is required to identify its active compounds.

Table.1 List of plants screened for antimicrobial activity (Moerman, 2009)

| S.No	Plants Name	Family	Common name	Medicinal use
1	*Astragalus crassicarpus*	Fabaceae	Milkvetch	Stimulant, hemostat, anticonvulsive, veterinary aid.
2	*Arctostaphyllus uva ursi*	Ericaceae	Bearberry	Dermatological aid, oral aid, antidiarrheal, analgesic etc
3	*Artemisia ludoviciana*	Asteraceae	Foothill sagewort	Gastrointestinal aid, antirheumatic, antidote
4	*Balsamorhiza sagittata*	Asteraceae	Arrow leaf Balsamroot	Dermatological aid, analgesic, gastrointestinal aid, pulmonary aid, burn dressing
5	*Caltha palustris*	Ranunculaceae	Yellow marshmerigold	Dermatological aid, cold remedy
6	*Centaurea stoebe*	Asteraceae	Spotted knapweed	Dermatological aid
7	*Chrysothamnus nauseosus*	Asteraceae	Rubber rabbitbrush	Dermatological aid and cold remedy, antidiarrheal
8	*Clematis ligusticifolia* Nutt	Ranunculaceae	Western white clematis	Analgesic, dermatological aid, cold remedy, veterinary aid
9	*Cleome serrulata*	Capparaceae	Rocky mountain beeplant	Gastrointestinal aid, dermatological aid, eye medicine
10	*Conyza Canadensis*	Asteraceae	Canadian horseweed	Antidiarrheal, orthopedic aid, antirheumatic, burn dressing, disinfectant
11	*Corallorhiza maculata*	Orchidaceae	Summer coralroot	Dermatological aid, cold remedy, pulmonary aid and blood medicine
12	*Cyanoglossus officinale*	Boraginaceae	Gypsyflower	Antihemorrhagic, cancer treatment, dermatological aid, kidney aid, tuberculosis remedy
13	*Geranium viscosissimum*	Geraniaceae	Sticky Geranium	Cold remedy, eye medicine, dermatological aid, Gynecological aid
14	*Glycyrrhiza lepidota Pursh*	Fabaceae	American Licorice	Antidiarrheal, gastrointestinal aid, ear medicine, pediatric aid, veterinary medicine, dermatological aid.

15	*Melilotus officinalis*	Fabaceae	Yellow sweetclover	Dermatological aid, cold remedy
16	*Monarda fistulosa*	Lamiceae	Wildbergamot beebalm	Dematological aid, ough medicine, throat aid, toothache remedy, gastrointestinal aid, eye medicine for sore eye.
17	*Oenothera biennis*	Onagraceae	Evening primerose	Dermatological aid, hemorrhoid remedy
18	*Onosmodium molle*	Boraginaceae	Smooth onosmodium	Veterinary aid, dermatological aid, antirheumatic
19	*Pediomelum argophyllum*	Fabaceae	SilverleafScrufpea	Used for fever, veterinary aid, used as laxative, dermatological aid
20	*Perideridia gairdneri* (Hook and Arn) Mathias (root)	Apiaceae	Giardner'sYampah	Antidiarrheal, cough medicine, dermatological aid, veterinary aid, diuretic and laxative
21	*Physialis virginiana*	Solanaceae	Virginia groundcherry	Used as stimulant
22	*Plantago rugelii*	Plantaginaceae	Blackseed plantain	Dermatological aid: poultice of fresh leaves applied to burn or any inflammation
23	*Psoralea argophylla*	Fabaceae		
24	*Quercus macrocarpa*	Fagaceae	Bur oak	Antidiarrheal, dermatological aid, pulmonary aid, gastrointestinal aid
25	*Rhus aromatic*	Anacardiaceae	Fragrant Sumac	Dermatological aid, urinary aid, antidiarrheal, cold remedy, oral aid, pediatric aid
26	*Senecio rapifolius*	Asteraceae	Openwoods ragwort	
27	*Symphocarpus albus*	Caprifoliaceae	Common snowberry	Diuretic and venereal aid, dermatological aid
28	*Tanacetum vulgare*	Asteraceae	Common tansy	Anthelmintic, dermatological aid, antidiarrheal, cold remedy

Table.2 List of plants with their MIC and MBC (minimum inhibitory concentration)

S.No	Plants Name	MIC g/ml plant extract in fresh wt	MBC g/ml Plant extract in fresh wt
1	*Centaurea stoebe*	0.062	0.5
2	*Clematis ligusticifolia*	0.031	0.5
3	*Monarda fistulosa*	0.125	0.5
4	*Onosmodium molle*	0.125	0.5
5	*Rhus aromatic*	0.25	0.5

Fig.1 Zone of inhibitions for the plant extract against *E. coli*. Positive control was gentamicin and negative control was ethanol. All ethanol controls had 0 mm Zone of Inhibition (ZOI)
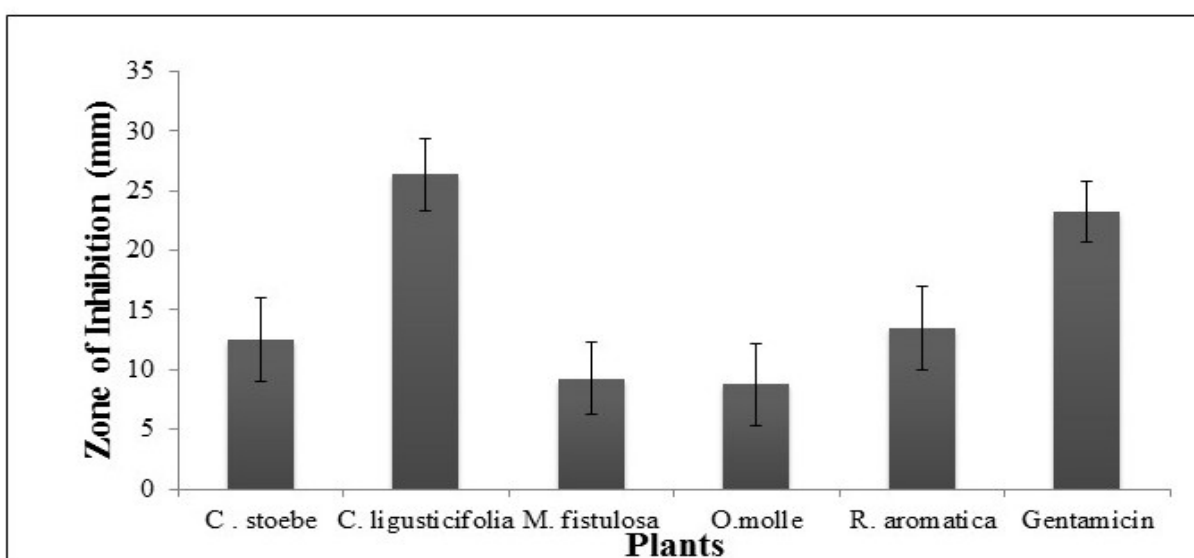
Fig.2 Incorporation of BrdU into DNA as a measure of cell growth, expressed as percent of incorporation into control cell DNA at the highest concentration of plant extracts, 0.5 g/ ml
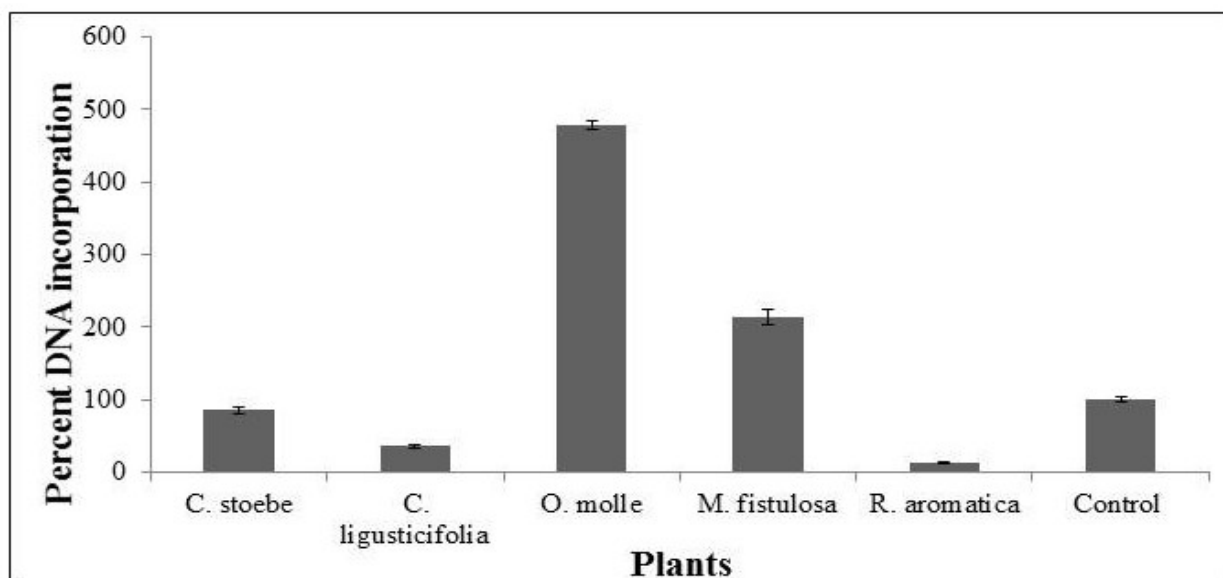
Fig.3 Cytotoxicity of *R. aromatic* extracts onIPEC-J2 cell growth
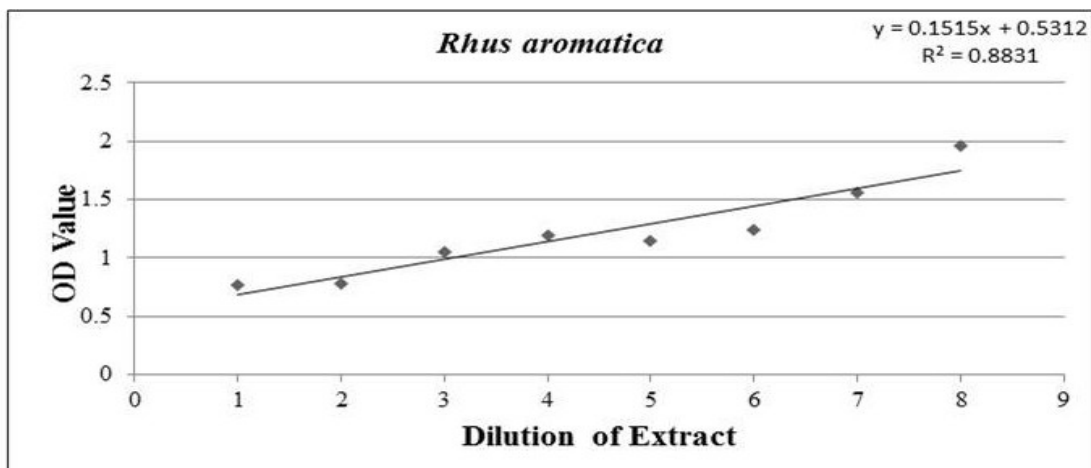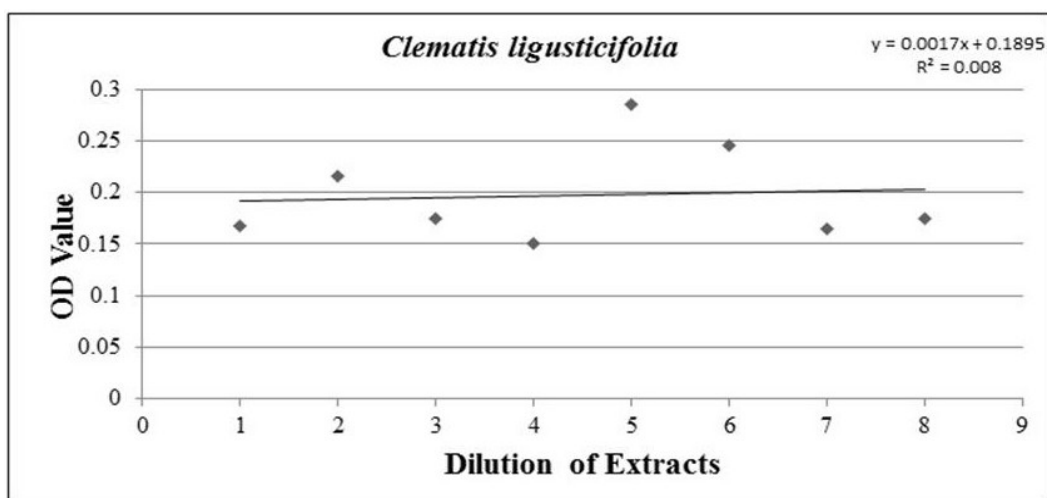

Fig.4 Cytotoxicity of *C. ligusticifolia* extracts onIPEC-J2 cell growth

Onosmodium molle (family- Boraginaceae) commonly known as false gromwell was used by the Native Americans as a dermatological aid, to treat lumbago and as veterinary medicine (Moerman, 2009). An infusion of *Onosmodium virginianum* root has been reported to help strengthening the renal apparatus (Cook, 1869). There is a paucity of data available in the literature concerning its mechanism of action. Our study showed antimicrobial properties of *Onosmodium molle* against *E. coli,* with no cytotoxicity effect on porcine intestinal epithelial cells. Because of it effect against *E. coli*, further investigation into the bioactive compounds of *O. molle* will be required.

This is the first report for *in vitro* antibacterial screening of Native South Dakota Plants against *E. coli*. The results suggest that further investigation into the antimicrobial activity of the extracts of *Clematis ligusticifolia*, *Monarda fistulosa*, *Rhus aromatica*, *Centaurea stoebe* and *Onosmodium molle* against *E. coli* is

warranted. It is also important to identify the secondary metabolites of these plants and their major active components having antimicrobial activity. Although monoterpene is the bioactive components in *M. fistulosa,* there is a paucity of information concerning the influence of growth environment and time of harvest on their antibiotic potential.

In this paper we showed that *M. fistulosa* and *O. molle* have inhibitory effect on *E. coli* but they are not toxic to the IPECJ2 cells. These two plants necessitate further study into their phytochemical inventory and their compounds that are active for treating infections. *C. ligusticifolia* and *R. aromatica* have promising antibacterial activity against *E. coli* but they also showed toxicity to the IPECJ2 cells *in vitro*. The application of these two plants should be tested *in vivo* to understand the toxicity effect for further medicinal use of these plants. The medicinal plants screened in this study may have metabolites which are effective for antibacterial activity and it needs to be further investigated.

Acknowledgement

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