EA	0	10.7±0.6	16±0.4	0	0	13.3±0.6	
A	0	0	0	0	0	12.7±0.2	
N	0			0			
P	14±0.70			14.7±0.62			
C	13±0.4	16.7±0.2	18±0.4	11±0.4	12.7±0.5	15.7±0.3	***S. typhi***
H	12.7±0.5	13±0.4	17±0.4	11±0.4	14±0.8	15.3±0.2	
Ch	13±0.40	19±0.40	21.7±023	0	16.7±0.2	18.3±0.2	
EA	11±0.40	15.3±023	18.3±023	0	11.7±0.8	14±0.4	
A	0	0	0	0	0	0	
N	0			0			
P	12.33±0.23			13.33±0.40			
C	0	0	0	0	0	0	***S. dysenteriae***
H	0	0	0	0	0	0	
Ch	0	0	0	0	0	0	
EA	0	0	0	0	0	0	
A	0	0	0	0	0	0	
N	0			0			
P	14.1±0.2			14.4±0.02			
C	11.7±023	14±0.40	17.3±0.5	10.2±0.2	13.3±0.2	16±0.40	***V. cholrea***
H	13±0.40	14±0.40	18±0.4	12±0.4	13.7±0.3	16.7±0.3	
Ch	13.3±0.6	16±0.40	18±0.4	13.3±0.3	14.7±0.2	17.7±0.2	
EA	0	0	12±0.40	0	11.3±0.6	13±0.40	
A	0	0	0	0	0	0	
N	0			0			
P	14±0.40			13.33±0.23			

TC = Test concentration; C = Crude; H = hexane; Ch = Chloroform; EA = Ethyl acetate; A = Aqueous; N = Negative control; P = Positive control

Hexane and chloroform fractions were the most effective ones against all the bacterial strains apart from *S. dysenteriae* which showed complete resistance. Hexane fraction showed maximum activity (23mm) against *P. aeroginosa* at 50mg/ml. Chloroform fraction showed highest activity against *E. coli, P. aeroginosa* (22mm) and *Aeromonas spp.* (>15 mm inhibition zones)., while the lowest activity was observed against *E. coli*. The aqueous fraction was marginally active against only two bacterial strains (*Aeromonas spp.* and *P. aeroginosa*). Hexane, chloroform and ethyl acetate fractions showed modest activity within the range of 14-16mm against *S. typhi* and *V. cholera* at high concentration (Table 1).

Ethyl acetate fractions showed excellent antibacterial activity against *E. coli* (>19mm), and good activities against the rest of the test bacteria. However, weak activity was observed for *Aeromonas spp*. The inhibitory zones produced by *E. obtusifolia* against the test bacteria are shown in Fig. 1.

Fig.1.1. *E. obtusifolia* against *Aeromonas spp.*

Fig.1.2. *E. obtusifolia* against *Aeromonas spp.*

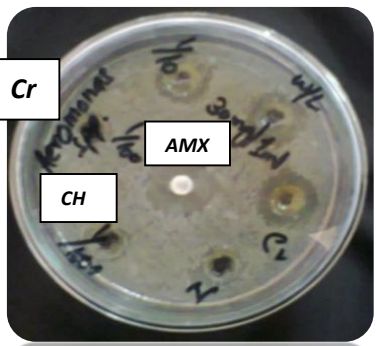

Fig.1.3. *E. obtusifolia* against *S. typhi*

Fig.1.4. *E. obtusifolia* against *Aeromonas spp.*

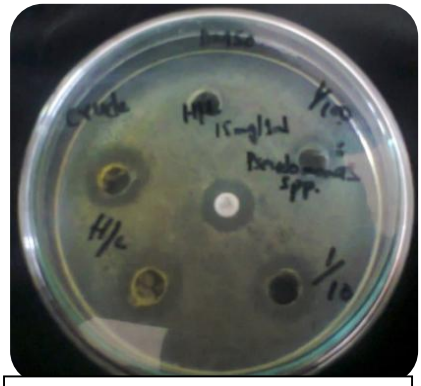

Fig.1.5. *E. obtusifolia* against *S. typhi*

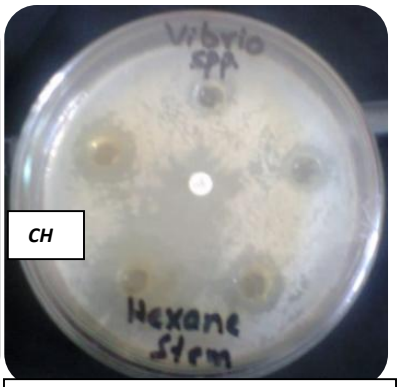

Fig.1.6. *E. obtusifolia* against *V. cholrae*

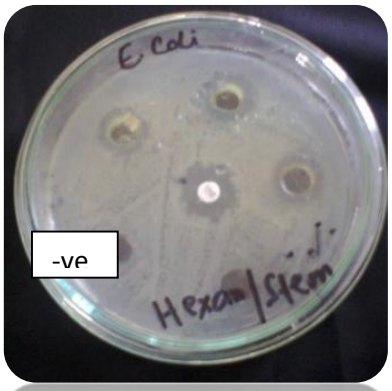

Fig.1.7. *E. obtusifolia* against *E. coli*

Fig.1.8. *E. obtusifolia* against *S. typhi*

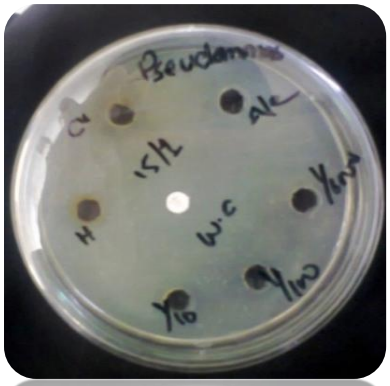

Fig.1.9. *E. obtusifolia* against *P. aeroginosa*

Fig 1: Activities of the *E. obtusifolia* against the selected pathogens

Antibacterial Activity of Stem Fractions of *Withania coagulans:* The stem extracts of *W. coagulans* showed antibacterial activity against all test bacteria. The highest zone of inhibition was shown by hexane extract (Table 2) at a conc. of 50mg/mL against *Aeromonas spp.* (>17 mm), while at concentration of 30 mg/ml it inhibited *E. coli* (>10 mm), *S. dysenteriae* (>12 mm), *V. cholrea* (>10 m m), and *Aeromonas spp.* (>15 mm).

Table 2: *Withania coagulans* stem and leaves extract inhibition zones (mean mm ± SEM) against selected Gram-negative bacter*ia*

	Stem extracts			Leave extracts			Test Bacteria
TC	***15mg/mL***	***30mg/mL***	***50mg/mL***	***15mg/mL***	***30mg/mL***	***50mg/mL***	
C	16.3±0.2	18.7±0.2	22±0.40	16±0.40	16.7±0.2	20.3±0.2	***Aeromonas spp.***
H	11.6±0.3	15±0.4	17.3±0.3	11±0.4	17±0.4	20.3±0.8	
Ch	0	15±0.4	20±0.4	0	0	15.3±0.8	
EA	0	12.3±0.6	17.6±0.6	0	12±0.4	15±1.0	
A	14±0.4	15±0.4	19±0.4	0	0	0	
N	0			0			
P	14.3±0.2			15.3±0.2			
C	11±0.40	16.3±0.6	21.7±0.6	14±0.4	15.7±0.2	16.6±0.3	***E. coli***
H	0	10±0.81	15.3±0.6	11±0.4	14.7±0.6	17±0.4	
Ch	0	0	11.3±0.6	10±0.4	14±0.4	19±0.4	
EA	0	0	12.6±0.6	0	11.7±0.6	15.3±0.8	
A	0	0	15.3±0.4	0	12.3±0.6	17±0.4	
N	0			0			
P	10.3±0.6			11±0.4			
C	10.7±0.4	15.3±0.2	16.3±0.6	0	11.6±0.3	16.6±0.2	***P. aeruoginosa***
H	0	0	12±0.40	0	10.66±0.5	15 ±0.40	
Ch	11.3±0.6	16±0.4	19.7±0.2	10±0.4	15±0.4	18± 0.4	

EA	0	12±0.4	15±0.4	0	12±0.4	16 ±0.4	
A	0	14±0.4	15.3±0.3	0	12±0.40	16.7±0.6	
N	0			0			
P	14.7±0.2			14.3±0.2			
C	0	14±0.4	17.6±0.2	11±0.4	12.3±0.4	15±0.4	***S. typhi***
H	0	0	0	0	11.3±0.6	14±0.4	
Ch	12.6±0.2	17.6±0.6	19.6±0.2	0	12±0.4	15±0.4	
EA	0	0	14.6±062	0	10.7±0.2	14±0.4	
A	0	11.3±0.6	14±0.4	0	10.7±0.2	14±0.4	
N	0			0			
P	13±0.4			12.7±0.62			
C	0	10.6±0.2	13.7±0.2	0	11.3±0.2	13.6±0.2	***S. dysenteriae***
H	0	12±0.4	13.6±0.6	0	11.3±023	14.7±0.3	
Ch	12.3±0.3	14.3±0.2	15±0.4	0	11.3±0.3	14.3±0.2	
EA	0	10±0.4	13.7±0.2	0	12.3±023	13.7±0.8	
A	0	0	0	0	13±0.4	15.3±0.3	
N	0			0			
P	12±0.4			13±0.4			
C	0	12.3±0.5	14.3±0.2	9.7±0.4	11.4±0.2	14±0.2	***V. cholrea***
H	0	10.3±0.2	12.7±0.2	10±0.3	12.7±0.2	15.7±0.40	
Ch	0	11.7±0.2	14±0.4	0	10.7±0.2	13.7±0.3	
EA	0	0	13.3±0.5	0	11.3±0.2	14.3±0.5	
A	0	10.7±0.2	12.330.6	0	11±0.4	14.3±0.2	
N	0			0			
P	12.3±0.2			12.3±0.2			

TC = Test concentration; C = Crude; H = hexane; Ch = Chloroform; EA = Ethyl acetate; A = Aqueous; N = Negative control; P = Positive control

Chloroform extract at a conc. of 50mg/mL inhibited very well the test *Aeromonas spp.* (>20mm). However, at 30mg/ml concentration *S. dysenteriae, S. typhi, V. cholrea, P. aeruginosa* and *Aeromonas spp* showed moderate inhibitory zones. Ethyl acetate extract at a conc. of 50mg/mL was inhibitory to *E. coli, S. dysenteriae, S. typhi, V. cholrea, P. aeruginosa* and *Aeromonas spp*. (Table 2). The aqueous extract showed inhibition of *Aeromonas spp.* and *P. aeruginosa* at 50mg/mL test concentration, but at lower concentrations the aqueous extract did not present a promising activity.

For crude extract (Table 2), the zone of inhibition at a conc. of 50mg/mL was, *E. coli* (>21 mm), *S. dysenteriae* (>13 mm), *S. typhi* (>17 mm) *V. cholrea* (>14 mm) *P. aeruoginosa* (>16 mm) and *A. spp.* (>22 mm). Crude extract also exhibited activities at a concentration of 30mg/ml against *E. coli* (>16 mm), *S. typhi* (>14 mm) *V. cholrea* (>12 mm), *P. aeruoginosa* (>15 mm), and *Aeromonas spp.* (>18 mm).

Antibacterial Activity of leaf Fractions of *Withania coagulans:* The antibacterial activity of leaves extract of *W. coagulans* also exhibited good inhibitions against test bacteria. The stem extracts showed better inhibitions compared to leaves extracts. The zone of inhibition for leaf crude extract at 50mg/mL concentration was, *A. spp.* (>20 mm), *E. coli* (>16 mm) and *P. aeruoginosa.* However, at the test concentration of 30 mg/ml moderate zones of inhibition were observed against all tested gastrointestinal pathogens (Table 2).

Hexane extract gave the highest inhibitory zones at a conc. of 50mg/mL against *Aeromonas spp.* and *E. coli* (>20 mm and 17 mm, respectively). In contrast moderate inhibitions were observed for *P. aeruoginosa, S. typhi, S. dysenteriae* and *Vibrio cholrea* (Table 2). At a conc. of 30mg/mL the activities were further reduced. Chloroform extract exhibited highest inhibitions against *E. coli* (>19 mm) and *P. aeruginosa* (>18 mm), while moderate inhibitions were observed against *Aeromonas spp., S. typhi, S. dysenteriae and V. cholera* (Table 2). The inhibitory zones are depicted in Fig. 2 against the test bacteria.

Phytochemical Analysis: The preliminary phytochemical investigations of *E. obtusifolia* and *W. coagulans* were performed, which shows the presence of glycosides, alkaloids, carbohydrates, flavonoids, steroids, saponins, protein, starch, phenol with anolides and tannins as main secondary metabolites.

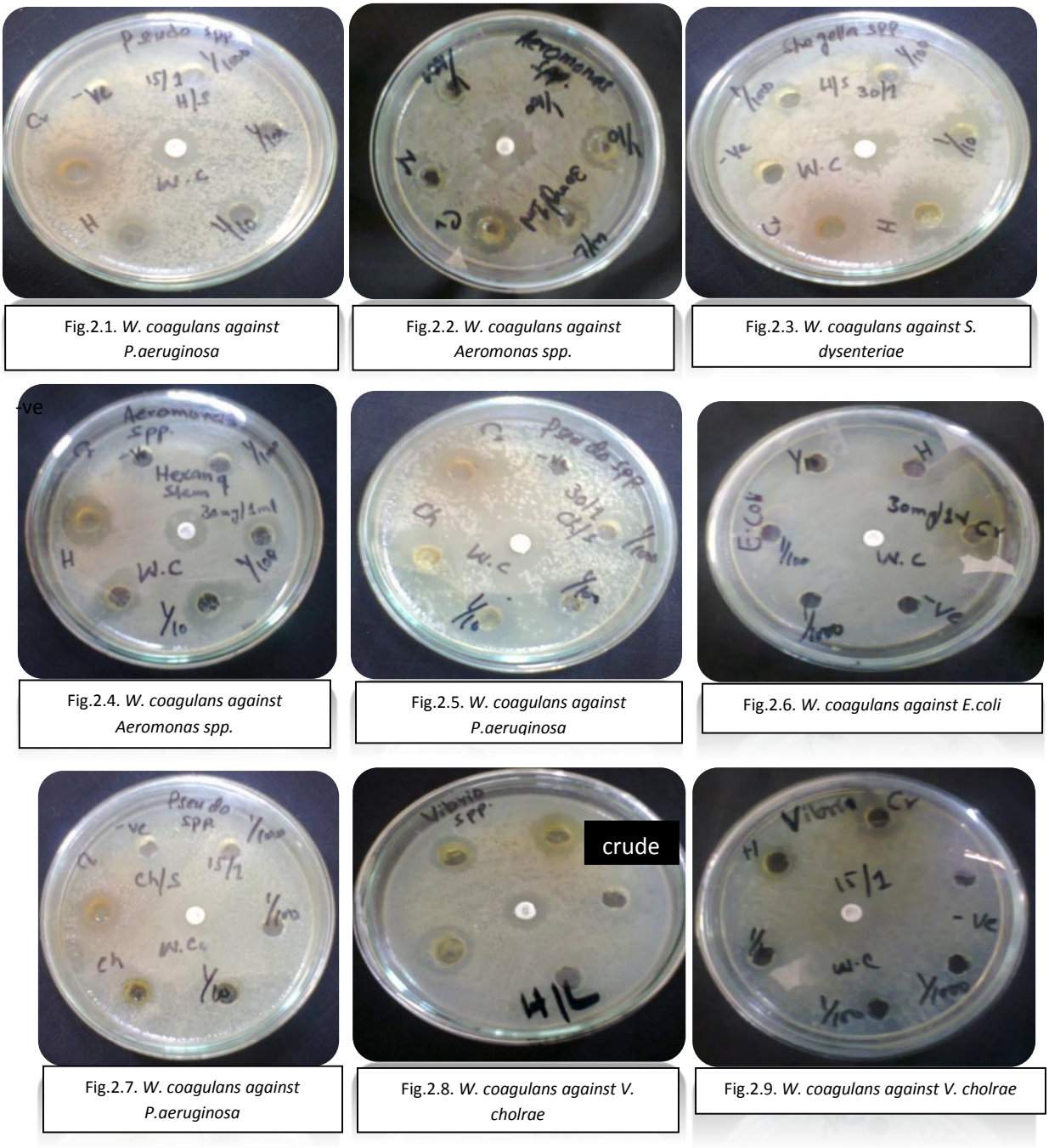

Fig.2.1. *W. coagulans against P.aeruginosa*

Fig.2.2. *W. coagulans against Aeromonas spp.*

Fig.2.3. *W. coagulans against S. dysenteriae*

Fig.2.4. *W. coagulans against Aeromonas spp.*

Fig.2.5. *W. coagulans against P.aeruginosa*

Fig.2.6. *W. coagulans against E.coli*

Fig.2.7. *W. coagulans against P.aeruginosa*

Fig.2.8. *W. coagulans against V. cholrae*

Fig.2.9. *W. coagulans against V. cholrae*

Fig 2: Activities of the *W. coagulans* against the selected pathogens

Phytochemical screening showed that alkaloids, steroids, saponins and flavonoids were present in higher amounts in stem of *E. obtusifolia* (Table 3), while carbohydrates, starch and protein were absent. Tannins were present in moderate amount. Glycosides and phenol were present in very low amount in crude stem extract. In leaves, steroids and tannins were present in higher amounts while, flavonoids, alkaloids and glycosides were present in moderate amounts. However, protein and starch were absent.

Table 3: Phytochemical Analysis of Extracts of *Ehretia obtusifolia* and *Withania coagulans*

S. No	Selected plants		*Ehretia obtusifolia*		*Withania coagulans*	
	Phytochemical	**Tests**	**Stem crude**	**Leaves crude**	**Stem crude**	**Leaves crude**
1.	Alkaloids	Mayer's	+++	++	-	-
2.	Carbohydrates	Molish's	_	+	++	+
3.	Flavanoids	Lead acetate	+++	++	+	+++
4.	Steroids	Salkowshi	+++	+++	_	_
5.	Glycosides	killer killani	+	++	+	++

6.	Saponins	Foam	+++	+	+	++
7.	Phenol	Ferric Chloride	+	+	+	++
8.	Tannins	Gelatin	++	+++	+	++
9.	Protein	Biuret's	_	_	+	++
10.	Starch		_	_	_	_
11.	Withanolides		-	-	+++	+++

*(+) Less amount, (++) moderate amount, (+++) higher amount, (-) absence of compound

The phytochemical analyses of *W. coagulans* indicate the presence of with anolides in higher content in both crude extracts. At the same time, steroids and starch were totally absent in crude extracts of leaves and stem. Proteins, glycosides, flavonoids, tannins and phenol were present in moderate quantities in stem extract while were found in low quantities in leaves crude extract, carbohydrates on the other hand were found in adequate quantities in leaves compared to stem extract (Table 3).

DISCUSSION

In the present study two medicinal plants *E. obtusifolia* and *W. coagulans* were screened for their activities against gastrointestinal bacteria. The crude extract was prepared in methanol and then fractionated into hexane, chloroform, ethyl acetate and water-soluble fractions. These plant extracts were tested in three different concentrations against six gastrointestinal pathogens, including *Aeromonas* spp. *E. coli, P. aeruginosa, S. dysenteriae, S. typhi* and *V. cholrae*.

The study demonstrates the potential of both the plants against selected pathogens and prospects of these plants for future use against the common gastrointestinal diseases. The stem extract of *E. obtusifolia* showed significant inhibitions against *Aeromonas* spp., *E. coli* and *S. typhi*. Hexane, chloroform and crude extracts of stem showed high zones of inhibition against *E. coli* and *Aeromonas* spp. while in case of *S. typhi* chloroform, ethyl acetate and crude extracts were found more effective. In case of *P. aeruginosa* chloroform and hexane showed the best activity while ethyl acetate and crude extract showed moderate zones of inhibition. The present study revealed that ethyl acetate exhibited moderate activity against *A.* spp. *E. coli* and *S. typhi*. The aqueous extract of *E. obtusifolia* stem part showed no activity against the selected pathogens in all test concentrations. In case of *S. dysenteriae* neither the stem nor leaves showed any inhibitory activity. Pharmacological screening of ethyl acetate soluble fraction revealed pronounced lipoxygenase inhibitory activity. Compounds methyl rosmarinate and rosmarinic acid are reported from the plant which has previously been reported to exhibit antihistamine, anti-inflamatory and lipoxygenase inhibitory activity [Mishra et al., 2013]. The presence of these phenolic compounds can be attributed to the antibacterial activities of the test fractions.

The crude leaves extract of *E. obtusifolia* was found most effective against *E. coli* and *P. aeruginosa*, while moderate activity was observed against *Aeromonas* spp. and *S. typhi*. All the sub fractions of the extracts showed significant activity against *E. coli, P. aeruginosa* and *V. cholrea*, while chloroform extract showed high inhibition against *S. typhi* and *Aeromonas spp*. These activities may be due to the presence of tannin, flavones and other phenolic components which are chloroform and ethyl acetate soluble. The leaves aqueous extract showed marginal activity against *Aeromonas spp*. and *P. aeruginosa*, and no activity were noted against *E. coli, V. cholrea* and *S. typhi* at any of the measured concentrations, which may be due to reason that all the phenolic and other non-polar and less polar compounds have been extracted in the organic solvents. The extracts of both stem and leaves gave almost similar results for *V. cholrae*. The chloroform extract of stem and leaves had stronger inhibitions compared to hexane, crude and ethyl acetate extracts. The results of the study suggest that the crude, chloroform extracts are more efficient against all the selected bacterial pathogens, followed by the hexane extract. Aqueous extract of leaves showed low inhibition or no activity against *these pathogens*.

W. coagulans have been reported for a number of pharmacological activities, found to exist in fruit, laves and roots [Nascimento et al., 2000]. In the present report, different extracts of *W. coagulans* stem part exhibited greater zones of inhibition against *Aeromonas spp*. Larger zone of inhibition was observed for crude extract of stem followed by chloroform, hexane, ethyl acetate and aqueous extracts. Moderate activity was noted against *E. coli* and *P. aeruginosa*. Crude extracts showed the highest zone of inhibitions against *Aeromonas spp*. and *E. coli*. The aqueous extract of the stem part showed inhibitions against all the pathogens, but the highest activity was recorded against *Aeromonas spp*, which may be due to the presence of some glycoside, while for the rest of test bacteria activity was moderate to low. Chloroform extract demonstrated strong activities against *Aeromonas spp. P. aeruginosa and S. typhi*. Our results are in agree-

ment with earlier finding that reported concentration of 15mg/mL *W. coagulans* had no activity against *S. typhi* [Maurya, 2010]. In the present study, stem's hexane extract showed no activity at any test concentration against *S. typhi*, while for *P. aeruginosa* weak inhibition was observed. However, moderate activities were observed for the rest of test bacteria. In case of ethyl acetate extract, moderate activity against all test pathogens was noted.

Similarly extracts from leaves of *W. coagulans* showed the highest zones of inhibition against *E. coli* and *P. aeruginosa* while moderated activities were observed against remaining test bacteria. The chloroform extract from leaves showed the highest activity against all the selected pathogens. The crude extract from leaves as well as hexane, chloroform, ethyl acetate, and aqueous fractions showed moderate zones of inhibition against *P. aeruginosa, S. dysenteriae, V. cholrae* and *S. typhi*. In case of crude and hexane extracts, highest activity was noted against *Aeromonas spp.*, however, no activity was seen in aqueous extract. The other fractions such as ethyl acetate and chloroform showed moderate activities. Chloroform showed highest activity against *E. coli* while rest of the fractions showed moderate zones of inhibition against the pathogen.

The earlier studies suggest that methanolic crude extract of *W. coagulans* exhibited moderate activity against *E. coli* and *P. aeruginosa* [Hoareau and J. DaSilva, 1999], which are in confirmation with our findings [Khandelwal, 2010]. In present study we also observed that ethyl acetate extract of *W. coagulans* has a good activity against *P. aeroginosa*. As far as concentration- effect was concerned, low activity was recorded at 15mg/ml concentration against all selected pathogens that got maximized at 50mg/mL concentration activity.

The qualitative phytochemical screening of the stem and leaves crude extracts of selected medicinal plants indicated the presence of various bioactive components. The results showed that in crude stem extract of *E. obtusifolia* alkaloids, flavonoids, steroids and saponins were present in high amount followed by tannins which were present in moderate amount. Phenol and glycosides were present in low quantities. In case of leaves crude extract steroids and tannins were present in high amount followed by flavonoids, alkaloids and glycosides that were present in a moderate amount. Similar results are already reported about the presence of alkaloids, flavonoids, tannins and phenols in all the extracts. Steroids were present in hexane and methanol extracts of *Ehretia laevis*, which is specie of *Ehretia* genus [Ullah et al., 2013]. While Carbohydrates, saponins and phenols were present in meager amount while proteins were absent in stem and leaves crude extracts. Carbohydrates were absent in stem crude extract. The presence of triterpenes, naphthoquinones, pyrrolizidine alkaloids, nitrile glycosides, phenolic glucosides, lignans and quinonoid xanthones are reported from some species of *Ehretia*, which supports our phytochemical screening results [Mishra et al., 2013].

In stem extracts of *W. coagulans* with anolides were present in a high amount while carbohydrates were found in moderate amount followed by flavonoids, tannins, glycosides, phenols, proteins and saponins which were present in meager quantities. The results of the presence of phytochemicals are in agreements to the previous reports about the presence of steroids, saponin, phenol, flavonoids, glucoside and tannins in various extracts of *W. coagulans* [Sudhanshu et al., 2012]. In crude leaves extracts withanolides and flavonoids were present in high amounts while saponins, tannins, glycosides, phenol, and proteins were found to be in moderate amounts. Our results are in confirmation to Mathur *et al.*, findings who reported the presence of saponin in extracts of *W. coagulans* [Sivasankari et al., 2013]. Carbohydrates were in trace amounts while steroids were absent in stem and leaves of *W. coagulans*, both of these observations are in agreement with earlier reports [Sivasankari et al., 2013].

CONCLUSION

The results showed that *Withania coagulans* as well as *Ehretia obtusifolia* have promising antibacterial potentials against notable gastrointestinal tract pathogens. *W. coagulans* showed the best activity against all test pathogens, while *E. obtusifolia* showed varying activities against all the test bacteria except *S. dysenteriae.* Phytochemical analysis also showed the presence of bioactive secondary components in both of these plants. This preliminary screening has opened the possibilities of the use of these plants in drug development for important pathogens of the gastrointestinal tract. Since the results of the phytochemical screening have shown that these plants are rich in alkaloids, tannin, with anolides, saponin, flavonoid, glycoside and phenols, further studies on isolation and characterization of the specific constituents are recommended.

Declaration of conflict of interest

None.

REFERENCES

Ahmad I. and A.Z. Beg, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens.