Available Online at

http://www.ijcpa.in

April-June 2020

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726; pISSN: 2395-2466

DOI: http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-7 | Issue-3 | Article ID: 0038

ANTIBACTERIAL ACTIVITY OF ROOTS OF *INULA RACEMOSA* AND *PHYTOLACCA ACCINOSA*

Maneesh Krashna*, Rohit Sarswat

OPJS University, Churu, Rajasthan, India.

***Corresponding Author:** Email: maneesh.j.krishna@gmail.com*

Received: 10 April 2020 / Revised: 24 June 2020 / Accepted: 25 June 2020 / Available online 30 June 2020

ABSTRACT

Plants used in traditional medicines contain a vast array of substances that can be used to treat chronic and infectious diseases and the present study was carried out with the same intent to appraise the possible medicinal value of Inula racemosa Hook and Phytolacca accinosa Roxb a threatened medicinal plant of Kashmir valley .The antimicrobial assay was carried out by Disc diffusion method. . The crude plant extracts of root part exhibited antimicrobial activity on majority of test organisms. The antimicrobial results revealed that the hydroalcoholic root extract of Inula racemosa and Phytolacca accinosa among the selected reference bacterial strains tested, the zone of inhibition was seen in concentration dependent manner on the four different Gram positive and four-gram negative strains.

Keywords *– Inula racemosa, Phytolacca accinosa, Gram positive, Gram negative, Bacteria.*

1. INTRODUCTION

Inula racemosa Hook F. [1,2] *and Phytolacca accinosa* Roxb [3,4] is an herbal medicinal plant belong to family *Asteraceae* and Phytolaccaceae respectively. *Inula racemosa* is stout shrub and has a leaf which are large in size in the racemose manner. The radical leaves of the plant are broad of about 20 x 40 cm and are elliptic lanceolate have long petioles. The root of the plant is stock branched, and the fresh roots are irregularly fusiform of about (20-25 x 5 cm). The number of roots sometimes found in collar zone although few occur usually in each clump and has a dull brownish skin have inside color yellowish.

Phytolacca accinosa is an herbaceous shrub [5], height up to 1.5m when grown in ground, Leaves are elliptical in shape have papery texture, tems color is green or reddish-purple, fleshy with the longitudinal grooves. Its flowers are white, clustered in the raceme inflorescences, often as long as the leaves. Fruits are small berries (7mm across), produced in infrutescences, ripen from green to purplish-black, containing red juice, and small 3-angled seeds, usually eaten and dispersed by seeds and roots are thick and fleshy [6]. Being a medicinal plant, both are used extensively for treating a variety of ailment. The present study deals with the comparative antimicrobial study of the root extract of *Inula racemosa* Hook F. and *Phytolacca accinosa* Roxb collected from different geographical zones of India.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh roots of *Inula racemosa* and *Phytolacca accinosa* medicinal plants were collected in the month of July 2014 from low altitude Gogaldor Tangmarg Kashmir, India. The roots of plants were used to prepare extracts for the study.

2.2 Chemicals

All the chemicals used were of AR grade from SD Fine chemical Ltd. (Mumbai, India).

2.3 Micro-organisms

The reference bacterial strains used in the study were obtained from Institute of Microbial Technology (IMTECH), Chandigarh (Table-1).

Table-1: List of bacterial strains used

Gram Positive Bacteria	Reference No.
Staphylococcus aureus	ATCC 6538
Enterococcus fecalis	MTCC 2729
Streptococcus mutans	MTCC 497
Micrococcus luteus	ATCC 6259
Gram Negative Bacteria	**Reference No.**
Escherichia coli	ATCC 2065
Pseudomonas aeruginosa	ATCC 741
Pseudomonas putida	ATCC 47054
Proteus vulgaris	ATCC 1335

2.4 Bacterial Strains

All the bacterial strains were maintained on nutrient agar (Hi Media) slants except *Streptococcus mutans* and *Micrococcus luteus* which were maintained on blood agar slants (Hi Media), sub cultured regularly (every 30 days or so) and after making their suspensions in the 10% glycerol and then were stored at 4°C as well as at - 80°C.

2.5 Preparation of Assay Media

The media mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. The prepared media was sterilized by autoclaving at 121 ^{0}C for 15 minutes.

2.6 Inoculum Preparation

- Take 4 ml of peptone water in a sterilize test tube and put an isolated colony with a sterilized straight wire into it.
- Mix well and incubate for 4 hr at 37 ^{0}C.
- Remove the test tube from the incubator and compare with the 0.5 Mc Farland standard that containing 0.5 ml of 1.75% (w/v) Barium Chloride dihydrate ($BaCl_2$. $2H_2O$) and 9.95 ml of 1 % (v/v) sulphuric acid (H_2SO_4), which gives turbidity equal to 1 to 2 × 10^8 CFU/ml.
- This bacterial suspension in the test tube is then used for antibacterial susceptibility testing.

2.7 Standard: Tigecycline was taken as standard.

2.8 Sensitivity of bacteria to different plant extracts by disc diffusion method

Here Kirby-Bauer (*Bauer* et al., 1966) method was followed. Sterile discs impregnated with antibacterial [7,8] agent place on culture plates and incubate the plates for overnight at 37 ^{0}C. Next day the zone of inhibition in diameter is measured around the disc to see the efficacy of different discs containing the different concentration of crude extract of the medicinal plant. The basic principle of this method is that a disc impregnated with antimicrobial agents absorbs the moisture from agar and antibacterial agent diffuses into the medium. The extraction rate of antibacterial agent is greater than the rate of diffusion from the disc.

All the extracts were tested against specific bacterial strains as follows:

- The bacterial colony picked up by inoculating wire and was passed into the normal saline tube. The turbidity of tube was compared with the 0.5 Mc Farland's opacity tube and was diluted or concentrated accordingly.
- The plates left at least 5-10 minutes for drying, after Whatman filter paper 6 mm discs (previously saturated with different concentrations of the extract) were put on the plates with the help of sterilized forceps. The final concentrations of the discs were 25-100µg/ml for bacterial strains.
- The antibiotics Tigecycline for bacterial strains were used positive controls.
- Then the plates were incubated in incubator at 37°C for 24 hours. Inhibition Zone of Diameter (IZD) around each disc was measured to the nearest mm with the help of ruler.

3. RESULTS AND DISCUSSION

3.1 Antibacterial activity of *Inula racemosa*

The antimicrobial activity of the extracts of *Inula racemosa* and *Phytolacca accinosa* were studied in different concentrations (mg/ml) (50, 75, 100) against eight reference bacterial strains which includes four Gram-positive (*S. aureus*: ATCC 6538, *E.fecalis*: MTCC 2729,S.mutans: MTCC 497,M.luteus: ATCC 6259) and four Gram-negative (E.coli: ATCC 2065, *P.aeruginosa*: ATCC 741, *P. putida*: ATCC 47054*, P. vulgaris*: ATCC 1335).

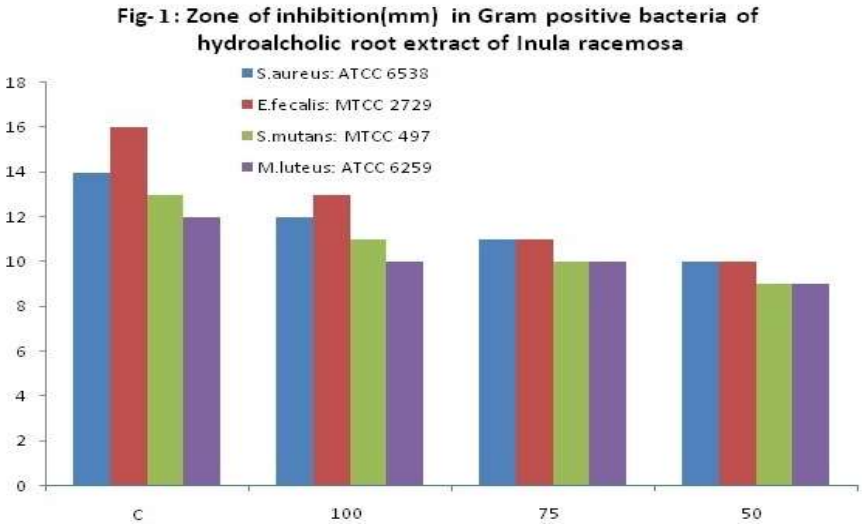
3.1.1 Antibacterial activity of *Inula racemosa* against gram positive bacteria

The antibacterial results revealed that hydroalcoholic root extracts of *Inula racemosa* were found to exhibit significant antibacterial activities against all tested bacterial strains. Among the four Gram positive bacteria tested at 100mg/ml *E. fecalis* (14 mm) and *S. aureus* (12 mm) gave the largest area of inhibition zone where as *S. mutans* (11mm) and *M. luteus* (10 mm) gave the least inhibition zone against root extract are summarized in Table-2. This shows that hydroalcoholic root extract of *Inula racemosa* had the antibacterial activity on increase in concentration while decrease with decrease in concentration. The Tigecycline (15 ug) was taken as control.

Table 2: Zone of inhibition (mm) in Gram positive bacteria of hydroalcholic root extract of *Inula racemosa*.

Conc. of extracts (mg/ml)	Zone of inhibition (mm)			
	S.aureus: ATCC 6538	***E.fecalis: MTCC 2729***	***S.mutans: MTCC 497***	***M.luteus: ATCC 6259***
Control (C)	14	16	13	12
100	12	13	11	10
75	11	11	10	10
50	10	10	9	9

Note: The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).



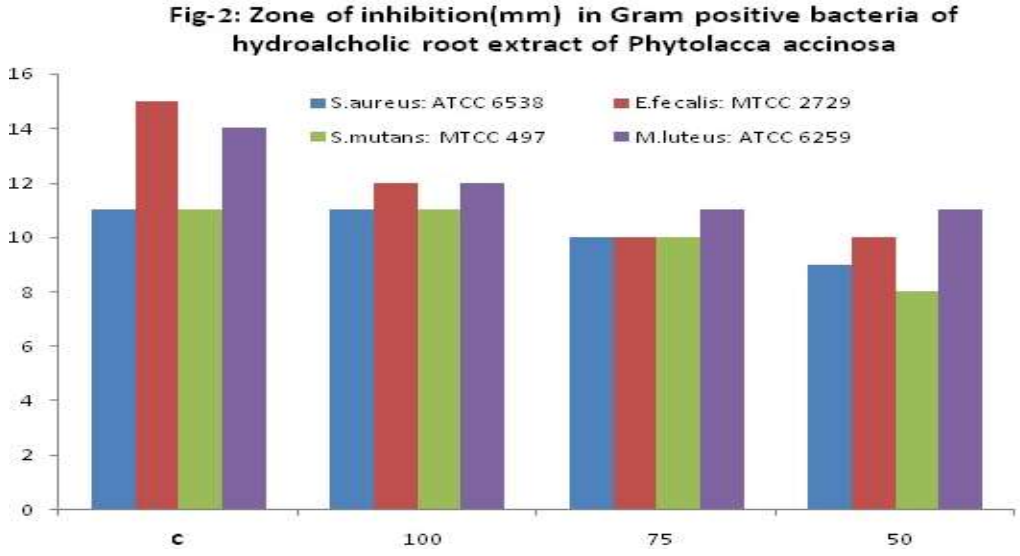
3.2 Antibacterial activity of *Phytolacca accinosa* against gram positive bacteria

The root extract of *Phytolacca accinosa* were tested against four Gram positive bacteria, the higher zone Inhibition diameter (IZD) was recorded on *E. feacalis* (12mm) and M. luteus (12mm), whereas *S. aureus* (11mm) and *S. mutans* (11mm) gave the least inhibition zone against hydroalcoholic root extract of *Phytolacca accinosa* are summarized in Table-3. This shows that hydroalcoholic root extract of *Phytolacca accinosa* had the antibacterial activity on increase in concentration while decrease with decrease in concentration. The Tigecycline (15ug) was taken as control.

Table 3: Zone of inhibition (mm) in Gram positive bacteria of hydroalcholic root extract of *Phytolacca accinosa*.

Conc. of extracts (mg/ml	Zone of inhibition (mm)			
	S.aureus: ATCC 6538	***E.fecalis: MTCC 2729***	***S.mutans: MTCC 497***	***M.luteus: ATCC 6259***
Conrtol (C)	11	15	11	14
100	11	12	11	12
75	10	10	10	11
50	9	10	8	11

Note: The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).



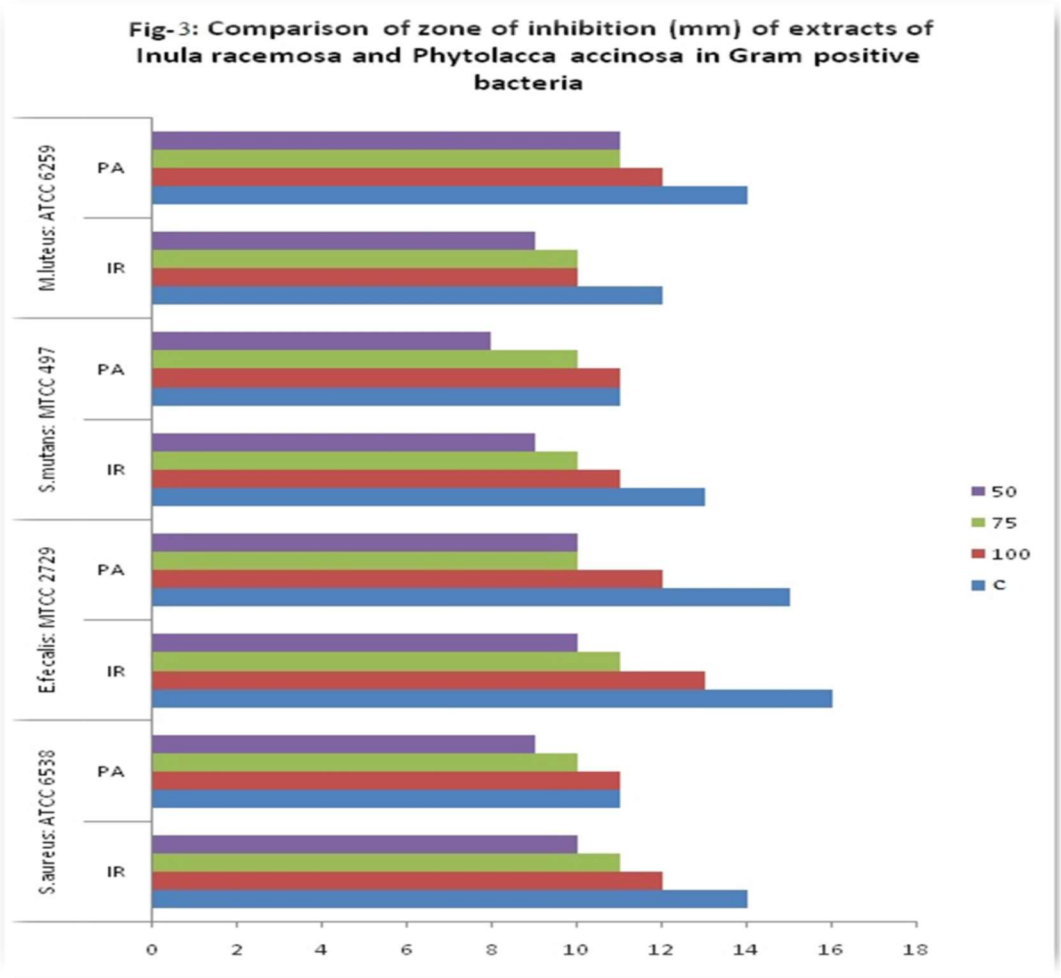
3.3 Comparative study of *Phytolacca accinosa* versus *Phytolacca accinosa* against gram positive bacteria

Comparative analysis reveals that the zone of inhibition of *Inula racemosa* and *Phytolacca accinosa* in four Gram positive bacteria tested at 100mg/ml, E.fecalis (13mm) followed by S.aureus(12 mm), S.mutans (11 mm) and M.luteus (10mm) gave the largest area of inhibition zone from extracts of *Inula racemosa* in comparison to extracts of Phytolaccaaccinosa which gives E. fecalis (12mm) followed by S.aureus and S.mutans (11 mm) which gave the least inhibition zone. However M.luteus (12mm) gave the large zone of inhibition from extract of Phytolacca acinosa than Inularacemosa which gives M.luteus (10 mm) are summerised in table-4.The result revealed that antibacterial activity of *Inula racemosa* against gram positive bacteria is better than the *Phytolacca accinosa*.

Table – 4: Comparison of zone of inhibition (mm) of extracts of Inula *racemosa* and *Phytolacca accinosa* in Gram positive bacteria.

Conc. of extracts (mg/ml	Zone of inhibition (mm)							
	S.aureus: ATCC 6538		*E.fecalis: MTCC 2729*		*S.mutans: MTCC 497*		*M.luteus: ATCC 6259*	
	IR	PA	IR	PA	IR	PA	IR	PA
Control (C)	14	11	16	15	13	11	12	14
100	12	11	13	12	11	11	10	12
75	11	10	11	10	10	10	10	11
50	10	9	10	10	9	8	9	11

Note: The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).IR: *Inula racemosa*; PA: *Phytolacca acinosa.* ATCC: American Type Culture Collection; MTCC: Microbial Type Culture Collection.



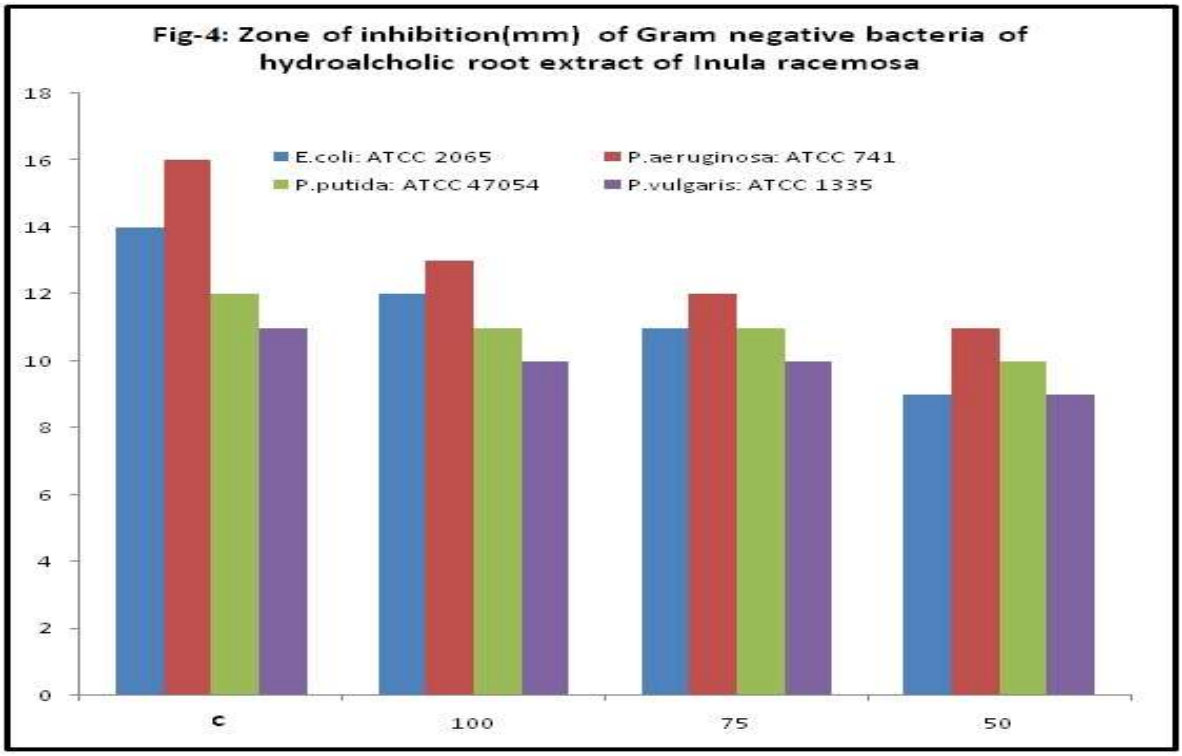
3.4 Antibacterial activity of *Inula racemosa* against gram Negative bacteria

The efficacy of solvent extracts of *Inula racemosa* among the four Gram negative bacteria tested showed varied levels of inhibition. At 100 mg/ml *P. aeruginosa (*13 mm) and *E. coli* (12 mm) gave the largest area of inhibition zone whereas P. putida (11 mm) and *P. vulgaris* (10 mm) gave the least inhibition zone against root extract are summarized in Table-5. This shows that hydroalcoholic root extract of *Inula racemosa* had the antibacterial activity on increase in concentration while decrease with decrease in concentration. The Tigecycline (15 ug) was taken as control.

Table 5: Zone of inhibition (mm) of Gram-negative bacteria of hydroalcholic root extract of *Inula racemosa*.

Conc. of extracts (mg/ml	Zone of inhibition (mm)			
	E. coli: ATCC 2065	***P. aeruginosa: ATCC 741***	***P. putida: ATCC 47054***	***P. vulgaris: ATCC 1335***
Control (C)	14	16	12	11
100	12	13	11	10
75	11	12	11	10
50	9	11	10	9

Note: The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).



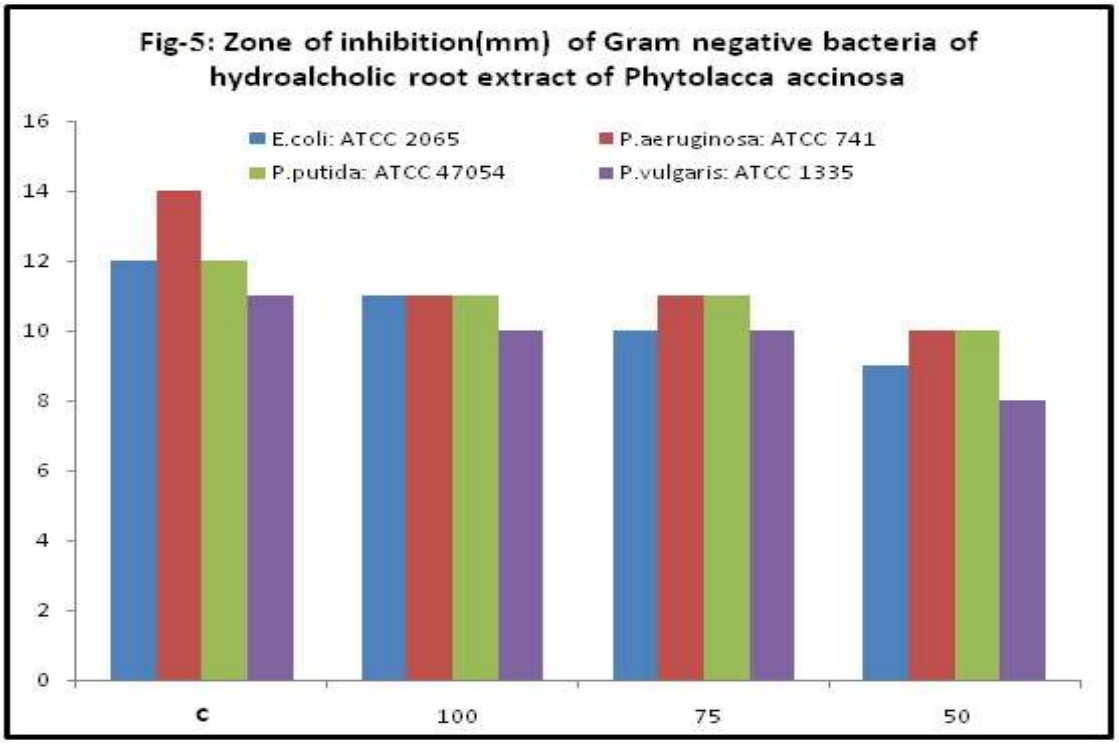
3.5 Antibacterial activity of *Phytolacca accinosa* against gram positive bacteria:

Among the four Gram negative bacteria tested, the highest Inhibition zone diameter (IZD) was recorded on *P. aeruginosa*, E.coli and *P. putida* (11mm) whereas *P. vulgaris* (10mm)gave the least inhibition zone against hydroalcoholic root extract *of Phytolacca accinosa* are summerised in Table-6. This shows that hydroalcoholic root extract of *Phytolacca accinosa* had the antibacterial activity on increase in concentration while decrease with decrease in concentration. The Tigecycline(15ug) was taken as control.

Table 6: Zone of inhibition(mm) of Gram-negative bacteria of hydroalcholic root extract of *Phytolacca accinosa*.

Conc of extracts (mg/ml	Zone of inhibition (mm)			
	E.coli: ATCC 2065	*P.aeruginosa: ATCC 741*	*P.putida: ATCC 47054*	*P.vulgaris: ATCC 1335*
Control (C)	12	14	12	11
100	11	11	11	10
75	10	11	11	10
50	9	10	10	8

The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).



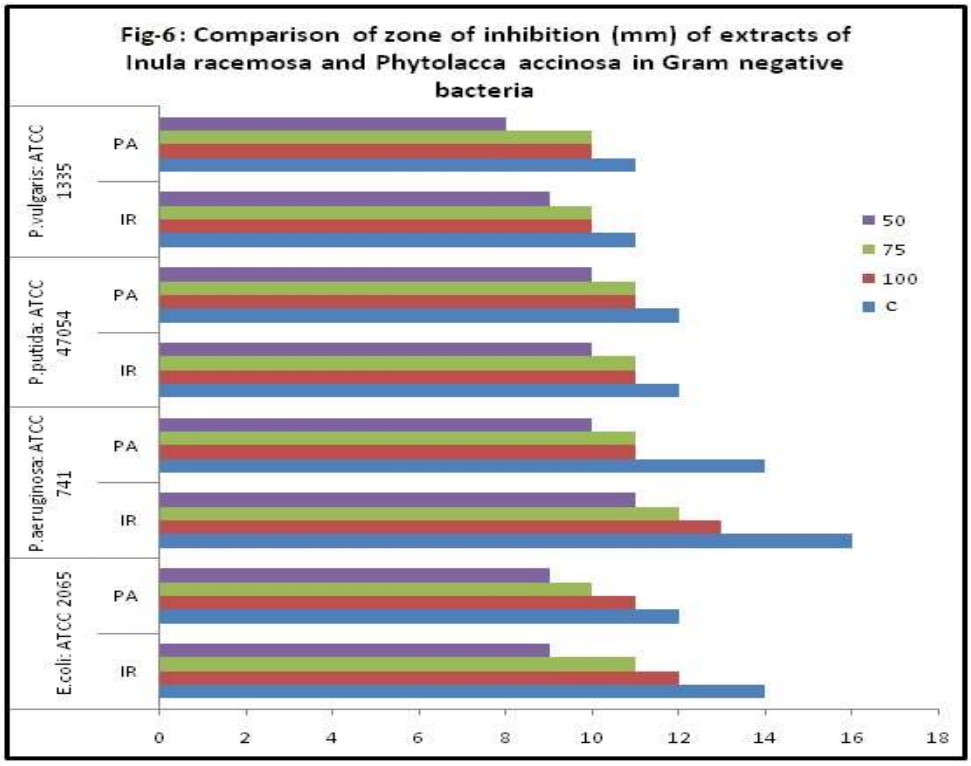
3.6 Comparative study of *Phytolacca accinosa* versus *Phytolacca accinosa* against gram negative bacteria

Comparative analysis reveals that the zone of inhibition of *Inula racemosa* and *Phytolacca accinosa* in four Gram negative bacteria tested at 100mg/ml, P.aeruginosa (13mm) followed by E.coli (12mm)gave the largest area of inhibition zone from extracts of *Inula racemosa* in comparison to extracts of *Phytolacca accinosa* which gives P.aeruginosa (11mm) and E.coli (11mm) gave the least inhibition zone. However the zone of inhibition of P.putida (11mm) and P.vulgaris (10mm)of both plants extracts are similar are summarized in Table-7.The result revealed that antibacterial activity of *Inula racemosa* against gram negative bacteria is better than the *Phytolacca accinosa*.

Table – 7: Comparison of zone of inhibition (mm) of extracts of *Inula racemosa* and *Phytolacca accinosa* in Gram negative bacteria.

Conc. of extracts (mg/ml	Zone of inhibition (mm)							
	E. coli: ATCC 2065		*P. aeruginosa: ATCC 741*		*P. putida: ATCC 47054*		*P. vulgaris: ATCC 1335*	
	IR	**PA**	**IR**	**PA**	**IR**	**PA**	**IR**	**PA**
Control (C)	14	12	16	14	12	12	11	11
100	12	11	13	11	11	11	10	10
75	11	10	12	11	11	11	10	10
50	9	9	11	10	10	10	9	8

Note: The value in table is by calculated the mean of experiment of three times and the data is presented in the table as Mean ± SEM. Control (Tigecycline).



4. CONCLUSION

In the present study, comparative analysis of both plants in among the Gram positive bacteria tested it was clear from the result that hydroalcoholic root extract of *Inula racemosa* exhibited good antimicrobial activity against *E. fecalis, S. aureus and M. luteus* gave the largest zone of inhibition in comparison to *Phytolacca accinosa* gave the slightly small zone, where as in *S. mutans, Phytolacca accinosa* gave slightly larger inhibition zone than *Inula racemosa*. In general, it was clear from the result that both medicinal plants do not show significant difference in antibacterial activity against gram positive bacteria as shown in fig. 3.

Similarly, among gram negative bacteria tested, it was clear from the result that the comparative analysis of both plant crude extracts, zone of inhibition was higher of *Inula racemosa* against *P. aeruginosa* and *E. coli* than Phytolaccaaccinosa. However, inhibition zone against *P. putida* and *P. vulgaris* of both crude extracts of plants was similar as shown in Fig-6. In general, it was clear from the result that the *Inula racemosa* exhibited good antibacterial activity against gram negative bacteria than *Phytolacca accinosa*.

In the present study, comparison of crude extracts of two selected medicinal plants from two different families. It was clear from the result that *Inula racemosa* exhibited greater antibacterial activity than the extract of *Phytolacca accinosa*. The difference may account for the higher antibacterial activity of *Inula racemosa* than *Phytolacca accinosa* are the nature of bioactive components.

REFERENCES

1. Manish Kumar Soni, Omprakash Sharma, Pushkarmoola (*Inula Racemosa* Hook. F.): A Ayurvedic Review, world journal of pharmaceutical and medical research, 2018,4(3), 199 - 201.
2. Kimothi G P. Pushkarmool (*Inula racemosa* Hook. f.) A Drug with Potential Bronchodilatory Properties, Info Ayurveda, 2014, 10(2); 20-22.

3. Yeung HW, Feng Z, Li WW, Cheung WK, Ng TB. Abortifacient activity in leaves, roots and seeds of *Phytolacca acinosa*. Journal of ethnopharmacology. 1987 Sep 1;21(1):31-5.
4. Ma XP, Zhang WF, Yi P, Lan JJ, Xia B, Jiang S, Lou HY, Pan WD. Novel Flavones from the Root of *Phytolacca acinosa* Roxb. Chemistry & Biodiversity. 2017 Dec;14(12): e1700361.
5. Aron PM, Kennedy JA. Flavan-3-ols: Nature, occurrence, and biological activity. Molecular nutrition and food research. 2008 Jan;52(1):79-104.
6. Singh A, Chandra R, Ali MN. Rate of extraction and phytochemical screening of selected medicinal herbs for herbal yoghurt. The Pharma Innovation. 2015 May 1;4(3, Part A):15.
7. Aziz, T., Mehmet, E.D. and Nazime, M. Antioxidant and antimicrobial activity of *Russula delica*: An edible wild mushroom. Eurasian Journal of Analytical Chemistry. 2007. 2: 54-66.
8. Baravalia, Y., Kaneria, M., Vaghasiya, Y., Parekh, J. and Chanda, S. Evaluation of antioxidant and antibacterial activity of *Diospyros ebenum* Roxb. Leaf (Ebenaceae). Turkish Journal of Biology. 2009, 33: 159-164.