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RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF *RICINUS COMMUNIS* LINN AND *THEVETIA PERUVIANA* SCHUM. OF KUMAUN HIMALAYA

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ABSTRACT

Objective: The aim of the present study was to evaluate the *Ricinus communis* and *Thevetia peruviana* for their phytochemical constituents and antibacterial activity. **Methods:** Successive hexane, chloroform, ethanol, methanol and aqueous plant extracts of these plants were screened for phytochemical constituents and antibacterial potential. *R. communis* bark and *T. peruviana* leaves were tested against a panel of microorganisms (*Bacillus subtilis, Escherichia coli*, *Agrobacterium tumefaciens*, *Xanthomonas phaseoli* and *Erwinia chrysanthemi*) by the disc-diffusion method. **Results:** Phytochemical analysis of *R. communis* extracts revealed the presence of saponins, phenols, flavonoids, quinines, and steroids. On the other hand, *T. peruviana* indicates that all extracts contained terpenoids, phenols, flavonoids, anthraquinones and free amino acids. The studied extracts of these plants displayed various degrees of antibacterial activities. The extracts of *T. peruviana* leaves showed the best spectra of activity against all tested microorganisms. The methanol extract was found to be most effective showing the largest zone of inhibition against *A. tumefaciens* (30±0.6 mm) and *E. coli* (28±0.3 mm). While methanolic extract of *R. communis* bark showed its highest inhibitory activity against *B. subtilis* (18±1.6 mm). The hexane extract of *R. communis* showed minimum antibacterial activity compared to all other extracts. **Conclusion**: The results of this study scientifically validate the inhibitory capacity of the above said medicinal plants attributed to their common use in folk medicine which might be useful in the development of new drugs based on natural resources.

Keywords: Phytochemicals, Antibacterial, Zone of inhibition, Plant extracts.

INTRODUCTION

Several plants and herbs that are used traditionally have potential antimicrobial properties and such reports persuade these to be phyto-antimicrobial agents [1]. During the last decade, reports of prevalence of resistance among microbes have increased astronomically but the development of antimicrobial drugs has not maintained the pace with the rate of development of drug resistance [2]. The amplification of diseases is largely due to indiscriminate use of antibiotics [3]. Recent studies have extensively addressed the dramatic increase of microbial resistance to antibiotics [4, 5]. Literature surveys show that plant-based drugs play a promising role in the treatment of infectious diseases [6, 7, 36]. The isolation of bioactive compounds such as phenols, flavonoids, alkaloids, terpenoids, quinones etc. for potential drug discovery has been extensively reported [8, 9, 10].

Ricinus communis L. (Euphorbiaceae) is also known as the castor oil plant. It is commonly found in both the tropical and temperate climates of the world [11]. The seeds of castor are the source of castor oil, used as a cathartic and also for lubrication and illumination.

Pharmacological activities of *R. communis* have studied by a few workers [12-18]. The leaf, root, and seed oil of this plant have been used for the treatment of the inflammation, liver disorders and Hypoglycaemic [19]. However, information on whole plant profiling for phytochemicals is very limited.

Thevetia peruviana belongs to the family Apocynaceae and it is commonly known as Yellow oleander and Lucky nut. It is an ornamental plant which grows in India, China, Australia. A decoction of the stem bark of *T. peruviana* is used as an antipyretic agent [20]. Flavone and Flavonol glycosides from the leaves of *T. peruviana* exhibit HIV-I reverse transcriptase and HIV-I intrase inhibitor activities [21]. It has also been regarded as a potential source of biologically active compound, namely insecticides, rodenticides, fungicides and bactericides [22]. Extracts of *T. peruviana* plant species contain glycosides, whose toxicity against snails, slugs, insects and humans has been documented [23-25].

Keeping above view in mind the phytochemical analysis and antimicrobial assessment of two selected angiospermic plants, *R. communis,* and *T. peruviana* were undertaken. This study on antimicrobial activity of two ethnobotanically known plants of Kumaun Himalaya would bridge the gap in this direction of existing knowledge.

MATERIAL & METHOD

Collection of plant materials

Two medicinal plants; *Ricinus communis* and *Thevetia peruviana* were selected for the present study. Several field surveys were arranged to study the natural habitats of selected medicinal plants and collection of plant material. The bark of *R. communis* (Fig. A) and leaves of *T. peruviana* (Fig. B) was collected from Rudrapur, Udham Singh Nagar, Uttarakhand and authenticated by the Department of Botany, Kumaun University, Nainital. The collected plants specimens were kept in the herbarium, Department of Botany, D.S.B. Campus, Kumaun University, Nainital.

Preparation of plant extracts

The collected plants materials were thoroughly washed and dried at room temperature (20 ± 2 ˚C), under the shade by keeping them onto the blotting papers. These samples were left for few days for drying however examined time to time to avoid any biological contamination. The blotting papers were changed at least once in 3-4 days. After complete the drying process, the samples were powdered with the help of a grinder. The prepared powders were filled in polyethylene zip bags and powders were used to evaluate the antimicrobial potential.

The extraction of plants compounds depends on upon the polarity of the solvent. Therefore, different polar and non-polar solvents such as; methanol, ethanol, chloroform, hexane, and distilled water were used for the extraction process. To prepare stock solution a fixed amount of powder was added to a fixed volume of solvents. The stock solutions were prepared in 250 ml flasks by dissolving 25g of powder into 150ml of solvents (w/v, 25g, and 150ml). These flasks were kept on a shaker for 6-8 hours at 180 rpm and then left for overnight. The extracts were filtered through Whatman filter paper no.1. The obtained filtrates were then evaporated by keeping them into water bath shaker at 40°C [26]. The evaporated condensed crude extracts were then dissolved with a respective solvent and used for the phytochemical and antibacterial assessment.

A B

Fig.1: A- Whole plant of *Ricinus communis* B- Close view of *Thevetia peruviana* leaves

Microorganisms used

Five (Gram +ve and -ve) bacteria (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No.40, *Agrobacterium tumefaciens* MTCC No.609, procured from Institute of Microbial Technology, Chandigarh, India and *Xanthomonas phaseoli* and *Erwinia chrysanthemi* obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India) were used in this investigation.

Screening of Antibacterial Activity

Antibacterial tests of selected microorganisms were carried out using disc diffusion method [27]. Nutrient agar plates (90mm size) were prepared and cooled down at room temperature (20 ± 2˚C). A small sterile cotton swab was dipped into the 24h old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. This process was repeated by streaking the swab 2 or more times rotating the plates approximately 60˚ each time to ensure even distribution of inoculum. After inoculation, the plates were allowed to dry at room temperature (20 ± 2˚C) for 15 min in the laminar chamber for settle down of inoculum. The filter paper discs (5mm) loaded with 40µl of extract were placed on the surface of the bacteria seeded agar plates and it was allowed to diffuse for 5 min than these plates were inoculated at 37 ± 1˚C for 18 h. Gentamycin (30 mcg) were placed into agar plates used as positive control and the respective solvent was also used as negative control. After 18h of inoculation, the diameter was observed for inhibition zones (measured in mm including disc size). Tests were performed in triplicates and observed values of Zone of Inhibition (ZOI) are expressed as a mean value with a standard error of means (SEM).

Qualitative phytochemical Screening

Preliminary phytochemical evaluation of all the extracts was performed by following standard methods of Harborne [28] to detect the presence or absence of certain bioactive compounds.

RESULTS

In the present study *R. communis* and *T. peruviana* were analyzed for phytochemical constituents. The screening was performed with methanol, ethanol, chloroform, hexane and aqueous extracts of both plants that make up a total of 10 extracts samples. The preliminary phytochemical screening of *R. communis* and *T. peruviana* revealed the presence of various secondary bioactive compounds as shown in table 1.

Table 1: Phytochemicals of *R. communis* bark extracts and *T. peruviana* leaves extracts.

S.No.	Phytochemical tests	Bark extracts of *R. communis*					Leave extracts of *T. peruviana*				
		M	E	C	H	A	M	E	C	H	A
1.	Molisch' s test (carbohydrates)	+	+	-	-	+	-	-	+	+	-
2.	Fehling's test (carbohydrates)	+	+	-	-	+	-	-	+	+	-
3.	Borntrager's test (glycoside)	+	-	+	+	-	-	+	-	-	+
4.	Keller- Killiani test (glycoside)	+	-	+	+	-	-	+	-	-	+
5.	Millon's test (protein)	+	+	+	-	+	+	+	-	-	-
6.	Xanthoproteic test (protein)	+	+	+	-	+	+	+	-	-	-
7.	Foam test (saponins)	+	+	+	+	+	-	-	-	-	-
8.	Salkowski test (terpenoids)	+	+	-	-	-	+	+	+	+	+
9.	Trichloroacetic acid test (terpenoids)	+	+	-	-	-	+	+	+	+	+
10.	Ferric chloride test (tannins)	+	-	+	-	+	-	-	-	+	-
11.	Ferric Chloride Test (phenol)	+	+	+	+	+	+	+	+	+	+
12.	Mayer's test (alkaloids)	-	+	+	-	+	+	+	-	-	-
13.	Dragendroff's test (alkaloids)	-	+	+	-	+	+	+	-	-	-
14	Alkaline reagent test (flavonoids)	+	+	+	+	-	+	+	+	+	+
15.	Shinoda test (flavonoids)	+	+	+	+	-	+	+	+	+	+
16.	NaCl test (starch)	+	-	-	+	-	+	+	-	+	-
17.	NaOH-HCl test (volatile oils)	+	+	-	-	-	+	-	-	+	-
18.	Aqueous HCl test (phlobatannins)	-	-	-	-	-	-	-	-	-	-
19.	Turbidity test (resins)	+	+	+	+	-	+	+	+	-	+
20.	Sulphuric acid test (quinones)	-	+	+	+	+	+	-	+	-	-
21.	Sudan-III reagent test (fat)	+	+	+	-	-	+	+	-	-	+
22.	Anthraquinone	+	-	-	-	+	+	+	+	-	+
23.	Free amino acids	-	-	-	-	-	+	+	+	+	+
24.	Sulphuric acid test (steroids)	+	+	+	+	+	+	-	-	+	+
	Total presence	**19**	**17**	**15**	**10**	**12**	**17**	**16**	**11**	**12**	**12**

+ present, - absent, M- methanol, E- ethanol, C- chloroform, H- hexane and A- aqueous

The methanol extract of *R. communis* demonstrated maximum occurrence of phytoconstituents (19/24), followed by ethanol extract (17/24), chloroform (15/24), aqueous (12/24) and hexane (10/24). Free amino acids and phalobatannin were not found in bark extracts of *R. communis* (Table-1).

The leaves extracts of *T. peruviana* demonstrated different metabolites presence with reference to the solvents of the plant (Table-1). The methanol extract of *T. peruviana* showed maximum secondary metabolites (17/24) followed by ethanol extract (16/24), hexane and aqueous (12/24 each) and chloroform extract (11/24). As evidence from the table-1, Saponin and phalobatannin were found totally absent in all the tested extracts of *T. peruviana* leaves. Comparatively, more variety of phytochemicals was noticed in methanol and ethanol extracts of both plants extracts. In this preliminary study, only phenols were detected in all extracts analyzed. Whereas, phalobatannin was totally absent in all extracts of both plants.

The present study also focused on the antibacterial activity of *R. communis* and *T. peruviana* extracts against 5 Gram-positive and Gram-negative bacterial strains using the disc diffusion method as shown in table 2-3 and plate 1-2.

Table 2: Antibacterial activity of *R. communis* bark extracts

Microorganisms	Zone of inhibition (mm)*					
	H	C	E	M	Aq	G
A. tumefaciens	7±0.0	6±0.0	19±1.3	12±1.3	na	25±0.3
B. subtillis	7±0.3	7±0.0	10±0.6	18±1.6	na	30±0.3
E. chrysanthemi	10±0.6	17±1.0	na	13±0.6	na	27±0.6
E. coli	8±0.0	6±0.3	18±1.0	15±1.7	na	28±1.0
X. phaseoli	na	10±1.3	20±0.0	15±1.6	na	26±0.7

***All the values are mean ± Standard Error of Mean (SEM) of three determinations, M – Methanol, E- Ethanol, C- Chloroform, H- Hexane, Aq- Aqueous extract, G- gentamycin (+ control) and na- not active**

Table 3: Antibacterial activity of different extracts of *T. peruviana* leaves

Microorganisms	Zone of inhibition (mm)*					
	H	C	E	M	Aq	G
A. tumefaciens	25±0.3	27±0.3	24±1.0	30±0.6	na	26±0.3
B. subtillis	21±1.2	23±1.2	22±0.3	26±0.3	na	28±1.3
E. chrysanthemi	18±0.6	24±1.6	23±0.6	24±0.8	na	26±0.3
E. coli	23±0.6	23±2.0	23±1.1	28±0.3	na	29±1.8
X. phaseoli	19±1.5	22±1.6	21±0.8	21±0.3	na	25±0.0

***All the values are mean ± Standard Error of Mean (SEM) of three determinations, M – Methanol, E- Ethanol, C- Chloroform, H- Hexane, Aq- Aqueous extract, G- gentamycin (+ control) and na- not active**

The antibacterial activity of *R. communis* bark and *T. peruviana* leaves was evaluated at 1000µg/ml in the present study and their potency were quantitatively assessed by Zone of Inhibition (ZOI). The present study indicated that all the tested extracts of *T. peruviana* leaves showed excellent inhibitory potential against all five tested microorganisms (Table-3). The results indicated that the methanol extract of *T. peruviana* leaves showed highest inhibitory activity by showed highest ZOI (30 mm) against *A. tumefaciens* followed by *E. coli* (28 mm), *B. subtilis* (26 mm), *E. chrysanthemi* (24 mm) and *X. phaseoli* (21 mm). On the other hand, the highest antibacterial potential of *R. communis* bark was found in ethanol extract (Table-2) against *X. phaseoli* (ZOI, 20 mm) followed by *A. tumefaciens* (19 mm) and *E. coli* (18 mm) but not active against *E. chrysanthemi*.

As evidence from table 2 and 3 that hexane extracts of both plants (*T. peruviana* and *R. communis*) were found less active among the all tested plant extracts. But hexane extract of *R. communis* leaves was found totally inactive against *X. phaseoli*. Aqueous extracts of both plants were also found completely inactive against all the tested microorganisms (Table- 2 & 3). It is interesting to note that methanol and chloroform extracts of *T. peruviana* leaves were found more active compared to used standard antibiotic (Gentamycin) especially against *A. tumefaciens* (Table 3).

DISCUSSION

Two angiosperms of Kumaun Himalaya *R. communis* and *T. peruviana* with five different solvent extracts (methanol, ethanol, chloroform, hexane and aqueous) were screened for the phytochemical and antibacterial properties. The methanol extracts of both plants showed the maximum of presence phytoconstituents compared to other extracts, which may be due the fact that methanol was comparatively more polar in nature. However, *R. communis* showed the presence of larger phytoconstituents which might be due to the physiological property of individual taxa. Thus, the preliminary phytochemical screening may be useful and lead to the detection of bioactive principles in drug discovery. Plants produce secondary metabolites not simply to adapt to their environment but also to resist themselves against several environmental stresses [29]. The secondary metabolites produced by the various adverse environmental conditions are flavonoids, alkaloids, polyphenols, terpenoids, quinones, steroids, polysaccharides etc [30]. Due to stressful climatic and geophysical conditions, Kumaun Himalayan region plants offer greater possibilities of having novel molecules and even larger quantities of active compounds [31, 32].

Relying upon the results obtained in the present investigation, it is clear that except aqueous extract almost all leaves extracts of *T. peruviana* were found positive for the pathogenic bacteria with variable effectiveness. The methanol fraction showed high inhibitory activity followed by chloroform and ethanol extract. This might be due to the presence of various substances which have activity against bacteria are more soluble in organic solvents than aqueous [33].

As evident from the available literature, *T. peruviana* and *R. communis* are documented for their use in remedies for various ailments. In a related study leaves extracts of *R. communis* have been reported for effectiveness against animal pathogenic bacteria [34].

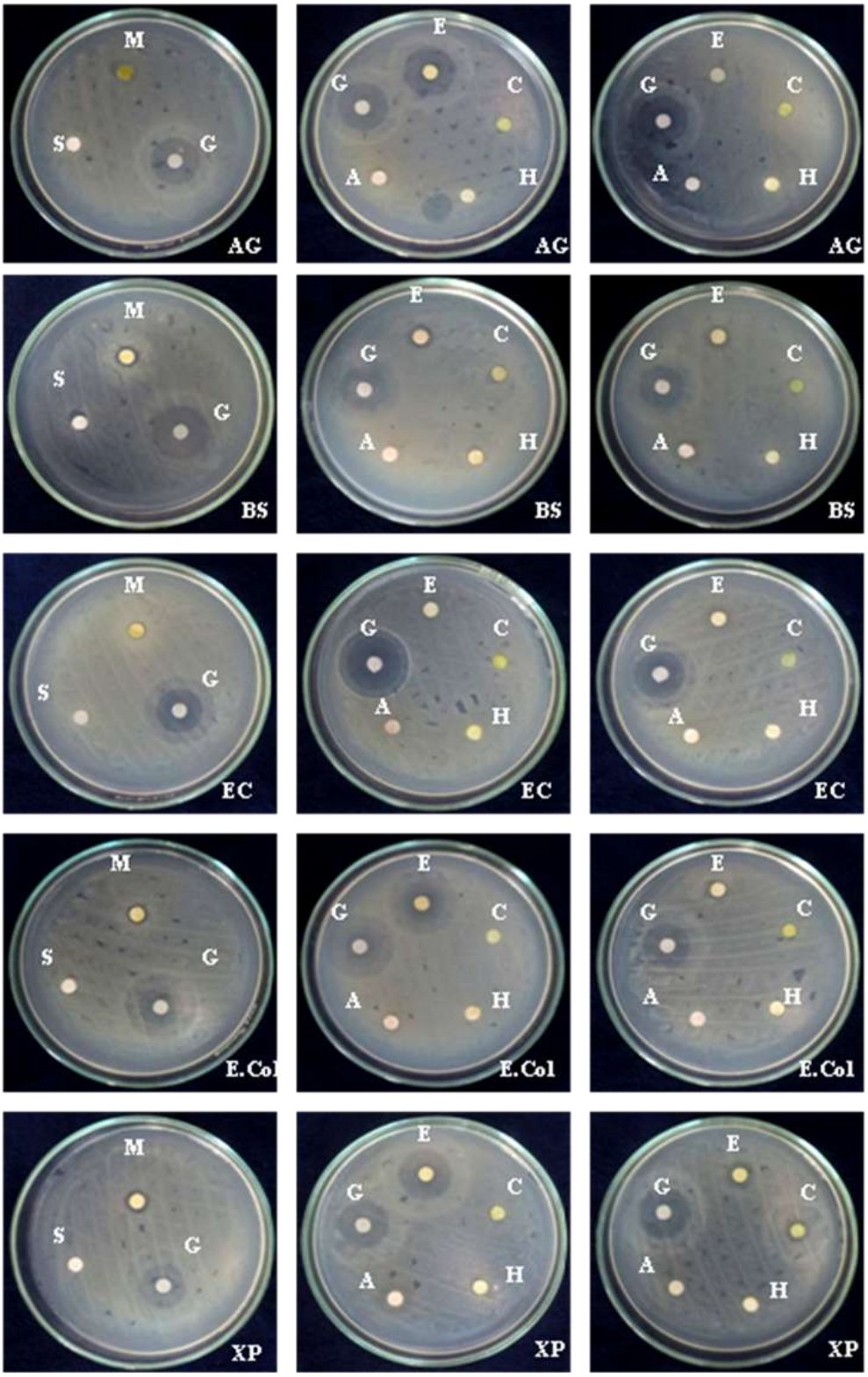

Plate 1: Antibacterial activity of *R. communis* bark extracts against some pathogenic bacteria. (E) - Ethanol extract (M) - Methanol extract, (C)- Chloroform extract (H)- Hexane extract AG- *Agrobacterium tumefaciens*, E.col- *Escherichia coli*, EC- *Erwinia chrysanthemi*, XP- *Xanthomonas phaseoli,* BS- *Bacillus subtilis,* (G) - Gentamycin (positive controls).

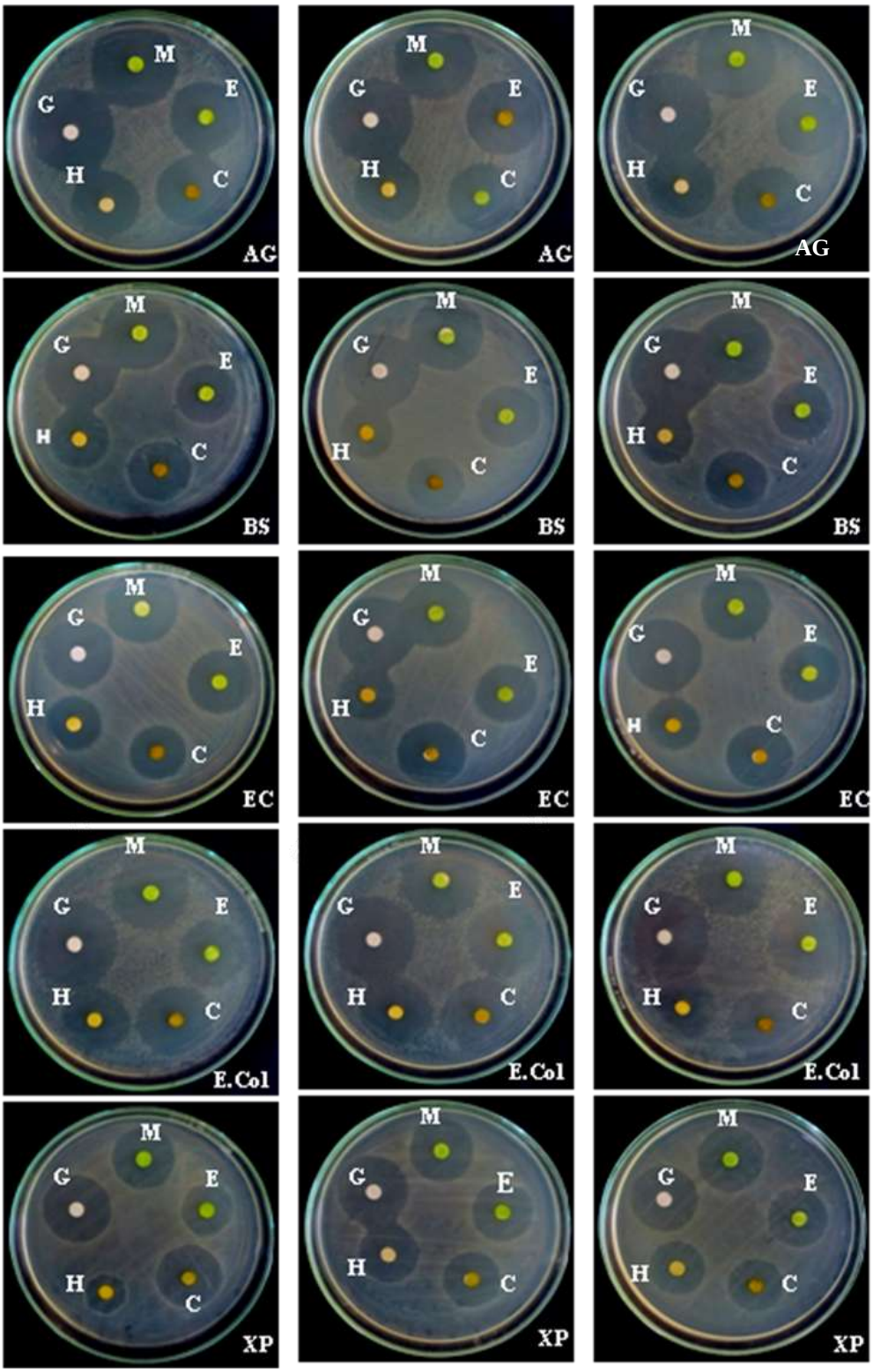

Plate 2: Antibacterial activity of *T. peruviana* leaves extracts against some pathogenic bacteria. (E) - Ethanol extract (M) - Methanol extract, (C)- Chloroform extract (H)- Hexane extract AG- *Agrobacterium tumefaciens*, E.col- *Escherichia coli*, EC- *Erwinia chrysanthemi*, XP- *Xanthomonas phaseoli,* BS- *Bacillus subtilis.* (G) -positive controls (Gentamycin).

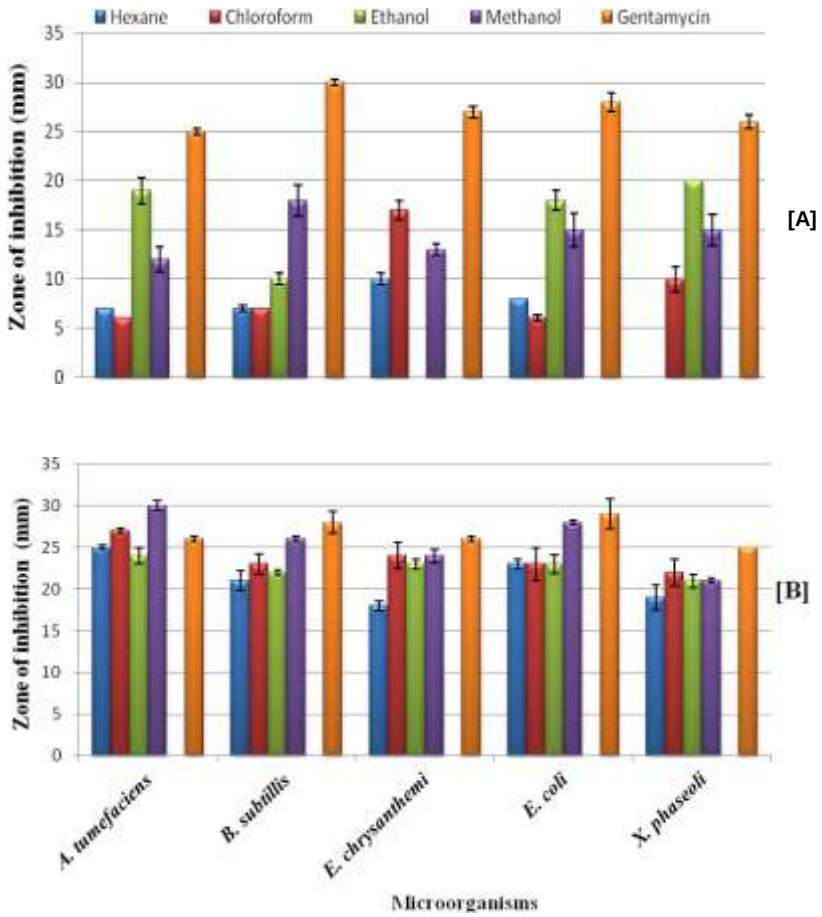

Fig.2: Comparison of antibacterial activity of *R. communis* bark extracts (A) and *T. peruviana* leaves extract (B)

The present findings support that ethyl acetate extract is the most effective fraction. Similarly, Kensa and Syhed also used crude extracts of *R. communis* leaves (ethanol, acetone, and hexane) against some animal pathogenic bacteria using disc-diffusion method [37] and found the ethanol extract of *R. communis* leaves was most effective against *E. coli*. The present study appears to be the first attempt to investigate the bark extracts of *R. communis* for its antibacterial potentiality against plant and animal pathogenic bacteria (*E. coli*, *A. tumefaciens*, *X. phaseoli* and *E. chrysanthemi*).

T. peruviana plants are toxic to most of the vertebrates as they contain cardiac glycosides [38]. The toxins are cardenolides called Thevetin A and Thevetin B (Cerebroside). The seed oil is also known for antifungal, antibacterial and antitermite properties [29]. Thus, the plant extracts of *T. peruviana* and *R. communis* were found positive for antibacterial potential against pathogenic bacteria (Plate 1-2) and comparative results of the antibacterial potential of different extracts of the plant are also represented in fig. 2. The active components usually interfere with growth and metabolism of microorganisms in a negative manner [39]. Antimicrobial properties of compounds in plant extracts are desirable tools in the control of infectious diseases microorganisms [35, 36].

It is interesting to note that the methanol and chloroform fractions of *T. peruviana* leaves showed better activity compare to standard antibiotic gentamycin used. It suggests that this plant contains more effective chemical components than the commercially available antibiotics to control the various plant and animal diseases causing microbes.

It is also noteworthy that the earlier workers investigated these plants against mostly animal pathogens whereas in this investigation antibacterial activity was tested against bacterial strains *X. phaseoli, A. tumefaciens* and *E. chrysanthemi* which are usually responsible for causing plant diseases like crown gall, leaf blight, leaf spot and rot diseases etc. The results of this investigation on antibacterial activities of *T. peruviana* leaves and *R. communis* bark extracts might be very useful in the pharmaceutical area.

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