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Antimicrobial activities and phytochemical analysis of ethanolic flower extract of *Thevetia peruviana* (Pers.) K. Schum (Thevetia Yellow)

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ABSTRACT

Thevetia peruviana (Pers.) K. Schum is widely grown as an ornamental and medicinal plant belongs to the family Apocynaceae and is commonly known as pili kaner. It is an evergreen and glabrous small tree .The cardiac glycosides obtained from bark, kernals and flowers are useful for heart diseases. The leaves are emetic and purgative. The ethanolic flower extract of Thevetia yellow was tested for antimicrobial activity against human pathogenic bacteria. Thevetia yellow flower extract showed strong antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa and Salmonella typhi.* The phytochemical and TLC analysis reveals the presence of alkaloids, essential oils, flavanoids, cardiac glycosides, phenolic compounds, phytosterols, saponins, tannins and terpenoids, which are mainly contributed to antimicrobial activity and medicinal utility of the plant. Thus Thevetia Yellow flowers may be utilized in the preparation of some newer antibiotics against tested microorganisms.

Key words: Antimicrobial activity, Cardiac glycosides, Phytochemical analysis, TLC (Thin Layer Chromatography), *Thevetia peruviana* (Pers.) K. Schum.

INTRODUCTION

The medicinal plants are a divine gift to us from 'mother nature' who has kept these green remedies in her plant kingdom for mankind to use and cure against various diseases and ailments. It is up to us to explore, seek, search and reap the benefits of this precious treasure. At present there are many valuable and lifesaving medicines obtained from plants. The plant world comprises a rich store house of biochemical that could be tapped for use as antimicrobial agent.

Thevetia peruviana (Pers.) is a small tree, with 3-6 m high belongs to the family Apocynaceae originally a native of America and West Indies. Leaves are simple, linear – lanceolate and whorled .Flowers 8-11 cm, medium, yellow,

solitary or in few flowered cymes. (Figure 1). All parts of this plant abound in a milky juice which is highly poisonous. (Chopra *et al*.,1984). The plant (Thevetia Yellow) is bitter, pungent, acrid, astringent to the bowels, useful in urethral discharges, worms, skin diseases, leucoderma, wound piles, eye trouble, itching, fever and bronchitis (Kirtikar and Basu,1981). The cardiac glycosides obtained from bark, kernals and flowers (Thevetia Yellow) are useful for heart diseases. (Prajapati *et al.,*2007) The root of this plant is made into a paste and applied to tumours. (Singh and Dey,2005). The leaves are emetic and purgative. Leaf decoction is given to prevent conception. The purified glycosides thevetin extracted from the seed is prescribed as a cardio tonic drug. Seeds used as an abortifacient and purgative in rheumatism and dropsy; also used as an alexeteric. Diluted latex is given to treat irregular menstruation. (Ambasta,1986; Kaushik and Dhiman ,1999; Retnam and Martin,2006).

MATERIAL METHODS

1. Collection of Plant Material

Plant materials (Flowers) of *Thevetia peruviana* (Pers.) were collected from Devi Ahilya Vishwavidyalaya campus, Indore. The collected plant materials were identified with the help of Flora of Madhya Pradesh. (Mudgal *et al*.,1997).

2. Extraction

To obtain ethanolic extract 100gm. of shade dried plant material was extracted with 500 ml. of ethanol (95%) in "Soxhlet Extraction Apparatus. Finally, the prepared plant material was macerated with water for 24 hrs. to obtain aqueous extract. Each extract was concentrated by distilling off the solvent (Kokate, 1994 and Kokate *et al*.,1993).

3. Preliminary Phytochemical Screening

The extract thus obtained was than subjected to preliminary phytochemical screening for identification of various plant constituents by methods suggested by (Finar, 1962; Farnsworth, 1996; Harborne, 1973; Harborne *et al.,*1979).

4. Thin Layer Chromatography (TLC)

Each ethanolic extract was than subjected to Thin Layer Chromatography by methods suggested by Kokate (1994), Stahl (1969), Wagner *et al*. (1984), Indhumathi and Mohandas (2013). The absorbent silica gel GF_{256} was coated to a thickness of 0.3 mm on clean TLC plates by commercial spreader. The plates were activated at 105°C for 30 minutes and used. Rf values were calculated. Various solvent systems were used to detect the phytochemical constituents. The selection of mobile phase depends upon, type of constituents to be analyzed. Here (10) different mobile phases were used.

5. Antimicrobial Testing

Each extract sample was tested for antimicrobial activity against human pathogenic bacteria by 'Cup Borer Method (Kavanagh, 1963; Cheesbrough,1993). The cultures of bacteria have been obtained from Microbial Type Culture, Gene Bank Chandigarh. The name and culture number of bacteria are as follows:

Gram-positive bacteria Gram-negative bacteria
Bacillus subtilis ATCC 6633.
Escherichia coli, MTCC 739
Staphylococcus aureus ATCC 9144
Klebsiella pneumoniae ATCC 33495
Salmonella typhi ATCC 10749
Pseudomonas aeruginosa ATCC 25668
Proteus vulgaris MTCC 1771

RESULTS & DISCUSSION

Phytochemical screening

The flower extract of Thevetia Yellow reveals the presence of alkaloids, flavanoids, glycosides-cardiac glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, saponins, terpenoids, proteins and amino acids was noted in the observation Table, while fixed oils, fats, gums and mucilages were found absent. (Table 1).

Thin Layer Chromatography (TLC)

In Thevetia Yellow flower extract maximum separation was found in Ethyl acetate: Benzene (2:1) and Ethyl acetate: Chloroform (6:4) mobile phases which are used for the detection of glycosides and terpenoids. The result of TLC analysis reveals the presence of alkaloids, amino acids, essential oils, flavanoids, glycosides, phenolic compounds, phytosterols, saponins, tannins and terpenoids.

Antimicrobial Testing

The ethanolic and aqueous flower extracts of Thevetia Yellow exhibits strong antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi.*

Table No.1: Phytochemical screening of ethanolic flower extract of *Thevetia peruviana* (Pers.) K. Schum [Thevetia Yellow]

S. No.	Plant Constituents Test/Reagents	Results
1.	**Alkaloids**	
	Mayer's reagent	+
	Dragendorff's reagent	+
	Hager's reagent	+
	Wagner's reagent	+
2.	**Carbohydrates**	
	Molish's reagent	+
	Benedict's reagent	+
	Fehling solution	+
3.	**Types of Carbohydrates**	
	Glucose	+
	Fructose	+
	Galactose	-
	Lactose	+
	Starch	-
4.	**Phytosterols**	
	Liebermann-Burchard's test	+
5.	**Terpenoids**	
	Salkowski reaction	+
6.	**Fixed oils and fats**	
	Spot test	-
7.	**Saponins**	
	Foam test	+
8.	**Phenolic compounds**	
	Ferric chloride solution	+
9.	**Tannins**	
	Lead acetate solution	+
10.	**Proteins**	
	Biuret test	+
	Xanthoprotic test	+
11.	**Amino acids**	
	Ninhydrin reagent	+
12.	**Gums and mucilages**	
	Alcoholic precipitation	-
13.	**Flavanoids**	
	Shinoda test	+
	Lead acetate test	+
14.	**Cardiac glycosides**	
	Killer kiliani test	+

+ Present, - Absent

Table No. 2: TLC observations of different phytoconstituents from ethanolic flower extracts of *Thevetia peruviana* (Pers.) Thevetia Yellow

S.No.	Name of the Phytoconstituents	Mobile phases	Visible Light			UV Light		
			Number of spots on TLC plates	Colour	Rf	Number of spot on TLC plates	Colour	Rf
1.	Alkaloids	$CHCL_3$: Methanol : Glacial acetic acid (83:17:10)	_	_	_	1 2	Dark Brown Dark Brown	0.75 0.96
2.	Amino acid	n-Butanol :Acetic acid:Water (4:5:1)	_	_	_	1	Dark Brown	0.66
3.	Essential oil	Hexane : Acetone (9:1)	1	Brown	0.20	1	Violet	0.20
4.	Flavanoids	Ethyl acetate :Methyl ethyl ketone :Acetic acid : Water(5:3:1:1)	_	_	_	1 2	Violet Violet	0.87 0.97
5.	Glycosides	Ethyl acetate: Benzene (2:1)	1	Brown	0.16	1 2 3 4	Brown Brown Brown Brown	0.23 0.36 0.86 0.98
6.	Phenolic Compounds	n - Butanol : Acetic acid : Water (35 : 5 : 12)	-	-	-	1 2	Violet Violet	0.68 0.97
7.	Phytosterols	P. ether : Ethyl acetate (7 : 3)	-	-	-	1	Violet	0.54
8.	Saponins	Chloroform : Methanol : Water (7:4:1)	1	Brown	0.86	1	Violet	0.86
9.	Tannins	Chloroform: Ethyl acetate: Ethanol (6:4:4)	-	-	-	1 2	Violet Violet	0.68 0.97
10.	Terpenoids	Ethyl acetate: Chloroform (6:4)	-	-	-	1 2 3 4	Violet Violet Violet Violet	0.17 0.42 0.82 0.98

Table No. 3*:* Antimicrobial activity of Thevetia Yellow flower extracts (ethanolic and aqueous) against gram positive and gram negative bacteria

S. No.	Extract used	Quantity of extract in ml.	Gram positive bacteria		Gram negative bacteria				
			Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Salmonella typhi*
			Average diameter of zone of inhibition in mm.						
1.	Ethanolic	.05	No Zone	12	12	12	10	10	No Zone
		.08	12	14	14	14	12	11	13
		.11	14	15	16	16	13	12	16
		.14	16	16	18	18	14	14	17
		.17	18	18	20	20	16	16	18
	R		0.894	0.990	1	1	0.990	0.985	0.855
2.	Aqueous	.05	No Zone	No Zone	10	10	No Zone	No Zone	No Zone
		.08	12	12	12	12	12	No Zone	10
		.11	14	14	14	14	13	12	12
		.14	16	16	16	16	14	15	14
		.17	18	18	18	18	16	16	16
	R		0.894	0.894	1	1	0.85	0.930	0.914

r = Correlation coefficient

r = +1 perfect positive correlation, r = -1 perfect negative correlation

Fig 1: *Thevetia peruviana* (Pers.) [Thevetia Yellow Flowers]

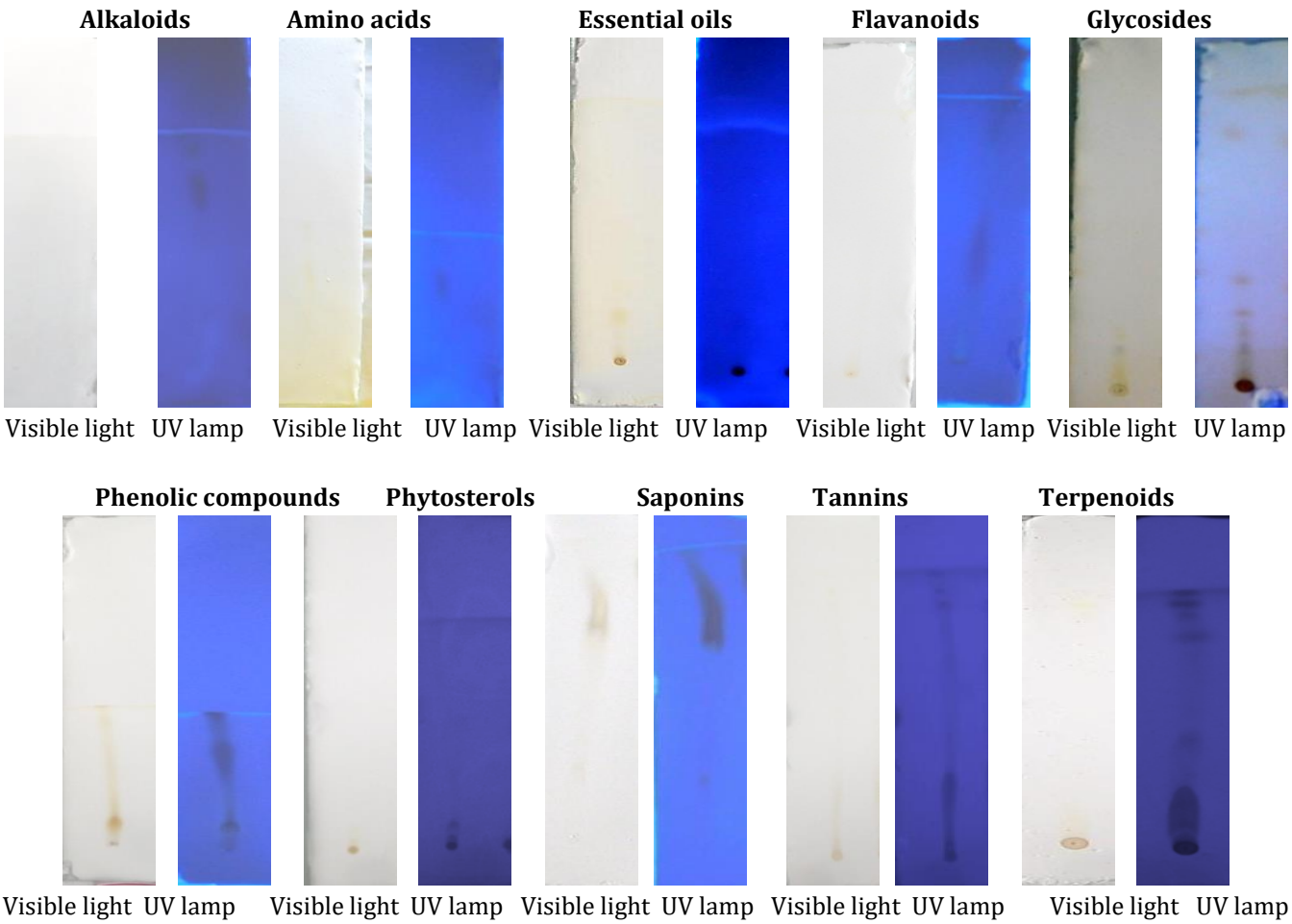


Fig.No.2: TLC observations of ethanolic flower extracts of Thevetia Yellow in different mobile phase.

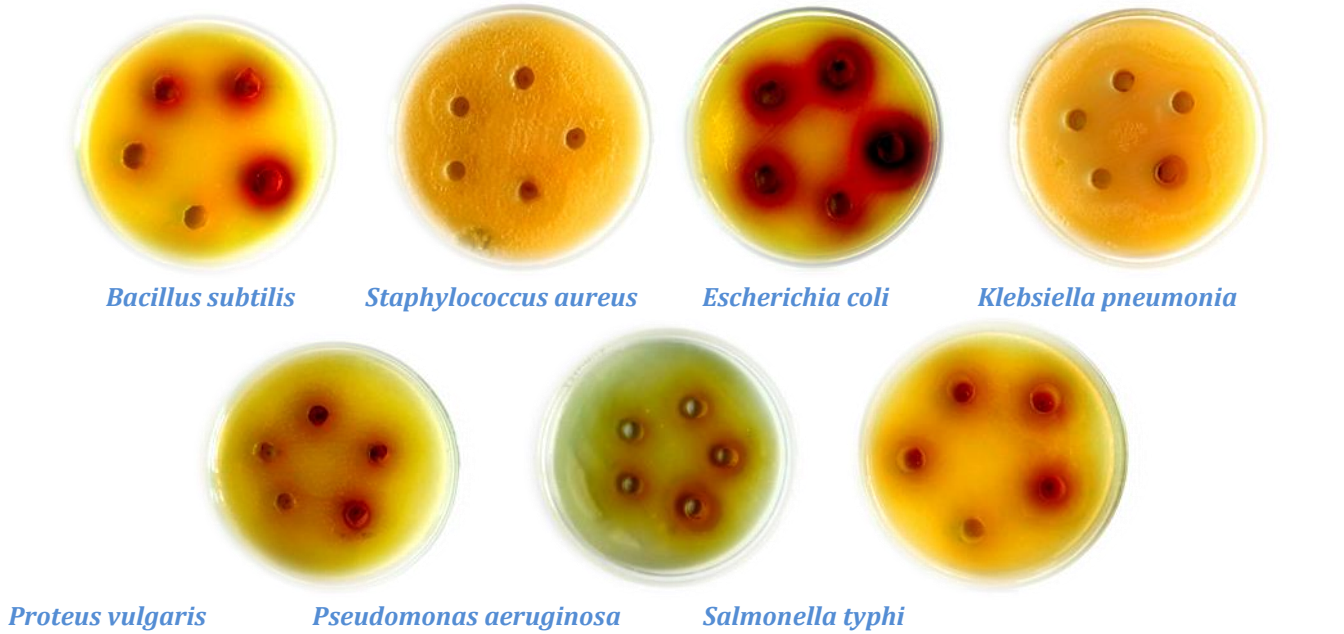


Fig.No.3: Antimicrobial activity of ethanolic flower extract of *Thevetia peruviana* (Pers.) K. Schum [Thevetia Yellow] against gram positive and gram negative bacteria.

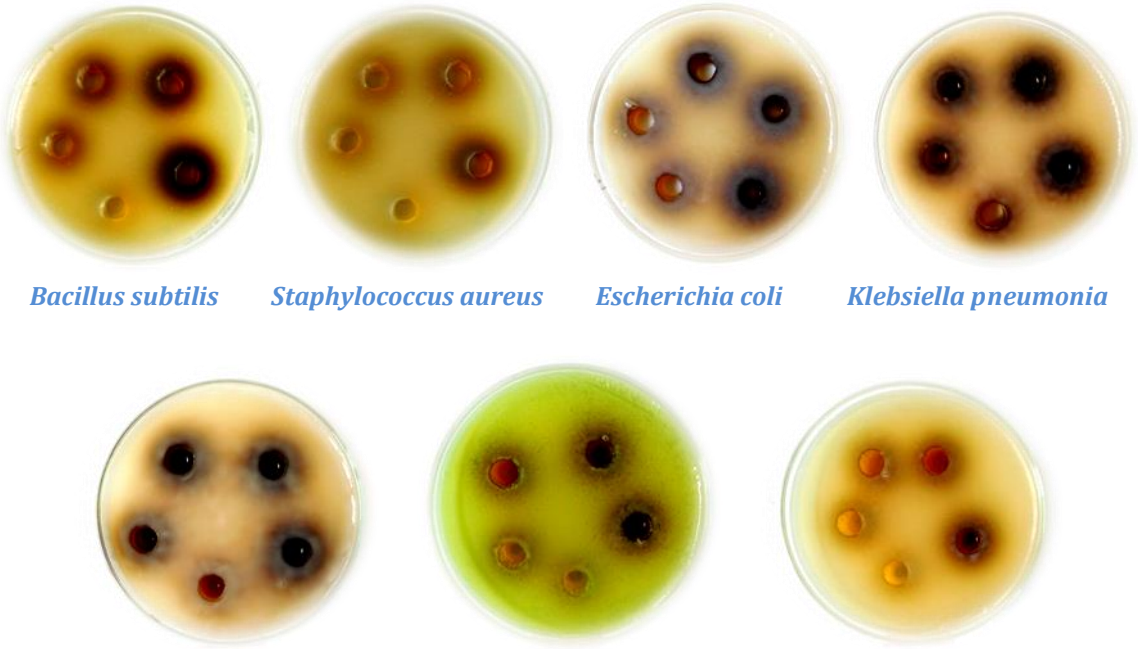
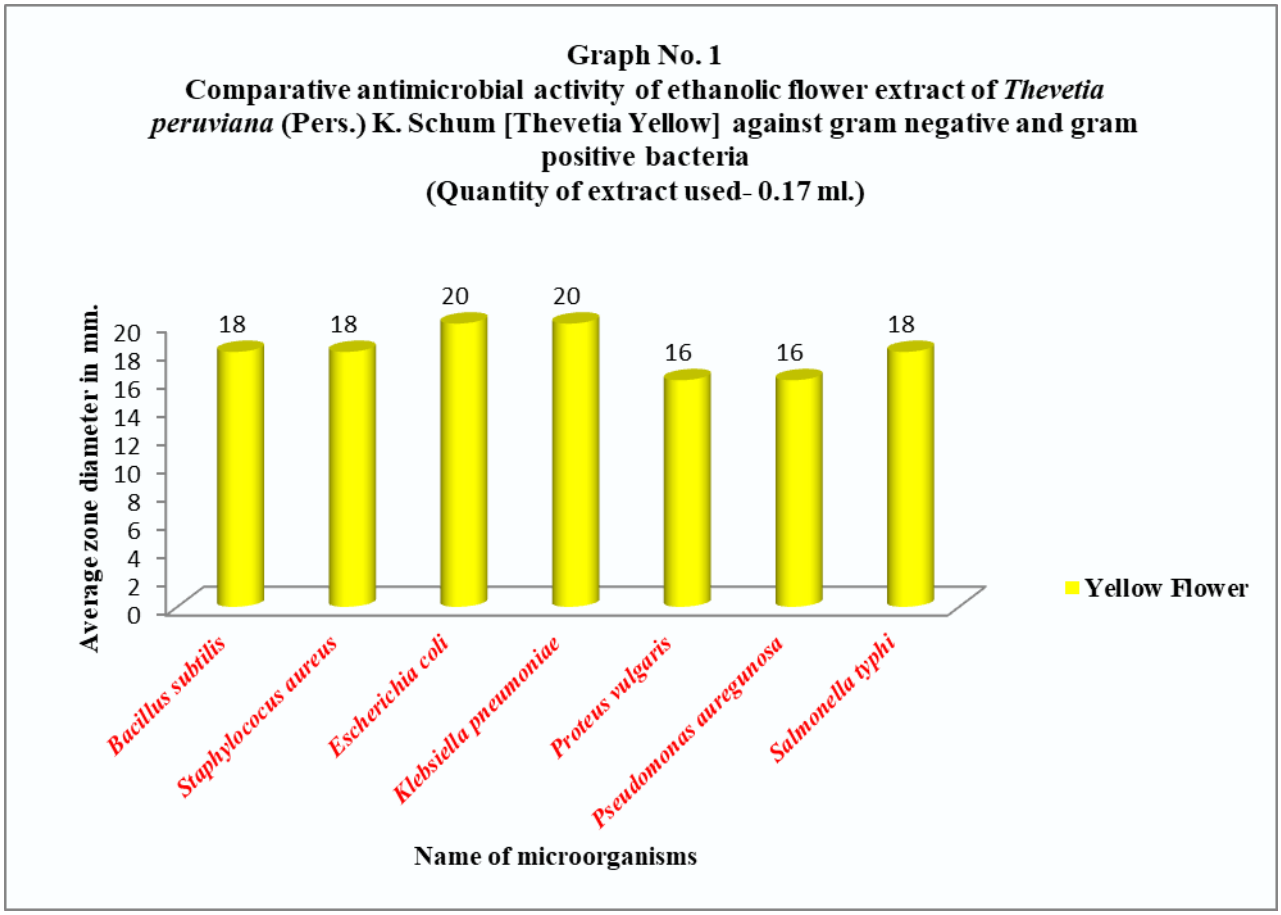
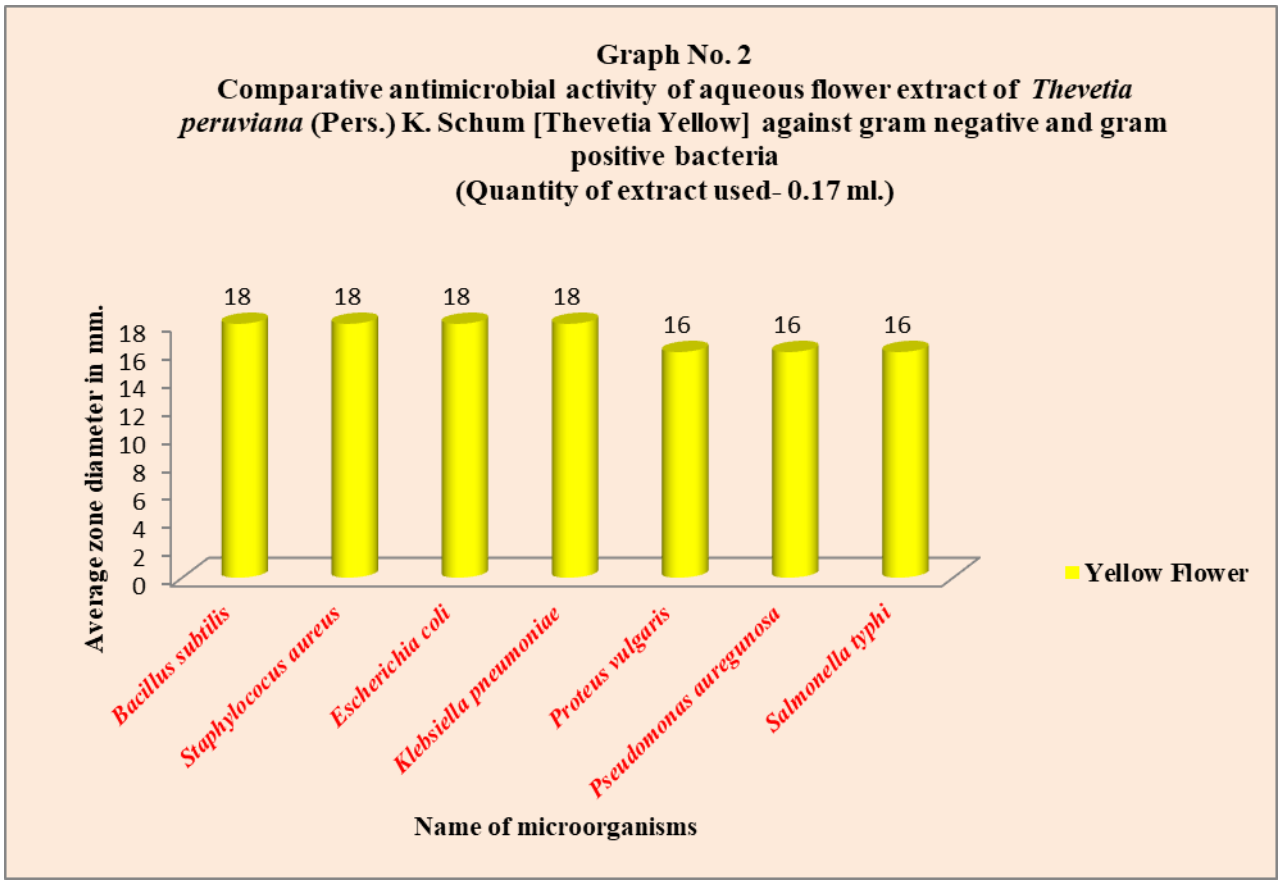


Fig.No.4: Antimicrobial activity of aqueous flower extract of *Thevetia peruviana* (Pers.) K. Schum [Thevetia Yellow] against gram positive and gram negative bacteria.





CONCLUSION

The flower extract of *Thevetia peruviana* (Pers.) K. Schum [Thevetia Yellow] showed strong antimicrobial activity against tested gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus* and gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi*. The results of preliminary phytochemical analysis reveals the presence of alkaloids, essential oils, flavanoids, cardiac glycosides, phenolic compounds, tannins, terpenoids, phytosterols and saponins. This was also confirmed by Thin Layer Chromatographic [TLC] analysis. So, this proves its correlation with antimicrobial activity, Thus Thevetia Yellow flowers may be utilized in the preparation of some newer antibiotics against tested microorganisms.

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Conflicts of interest: The authors stated that no conflicts of interest.

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