International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

ISSN 2347-2677
IJFBS 2018; 5(6): 125-139
Received: 22-09-2018
Accepted: 23-10-2018

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Phytochemical screening, sugar content, total protein and antimicrobial activity of three important medicinal plants

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Abstract
The present study was carried out to find out the antimicrobial activity of methanol extracts of three important medicinal plants respectively *Putranjiva roxburghii*, *Achyranthus aspera* and *Citrullus colocynthis* showed activity against all the selected bacterial strains. Methanol extracts of *Achyranthus aspera* and *Citrullus colocynthis* showed min. zone of inhibition against the selected gram positive*S. Haemolyticus* (+ve) and maxi. Zone of Inhibition for gram negative bacteria *K. pneumonia* (-ve). Methanol extracts *Putranjiva roxburghii* of showed min. zone of inhibition against the selected gram positive*S. Haemolyticus* (+ve) and maxi. Zone of Inhibition for gram positive bacteria *B.cereus* (+ve). Methanol extracts of three important medicinal plants respectively *Putranjiva roxburghii*, *Achyranthes aspera* and *Citrullus colocynthis* showed min. zone of inhibition against the selected gram positive*S. Haemolyticus* (+ve). On comparing MIC concentration of different plant extracts with Standard Chloramphenicol (100 µg/ml), it was observed that methanol extracts of *Achyranthus aspera* and *Citrullus colocynthis* gave best result against *K. pneumonia* (-ve), whereas Methanol extracts *Putranjiva roxburghii* gave best result against the selected gram positive (+ve) bacteria *B. cereus*. Antimicrobial analysis was done by using agar well diffusion method against bacterial pathogens. MIC value was determined by using micro broth dilution method. The phytochemical analysis showed the presence of tannins, flavonoids, saponins, terpenoids, and cardiac glycosides.

Keywords: antimicrobial, methanol extract, pathogen, phytochemical analysis, secondary metabolites

1. Introduction
General Introduction
Bacterial infections are an emerging problem worldwide, especially in developing countries, such as India (Sanders, & Riddle M.S *et al*., 2008) [1]. Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and reemerging bacterial pathogens to the available modern drugs or antibiotics (Franklin, *et al*., 2002).

In the developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicines for the treatment of common infections (Fabricant D.S, & Farnsworth N.R. *et al*., 2001) [4]. These plants are ingested as decoctions, teas and juice preparations to treat various types of infections. They are also made into practice and applied directly on the infected wounds and burns. These communities have a reduced risk from resistant pathogens than people from urban areas treated with traditional antibiotics. Moreover, if they are treated in a hospital the chance of contracting a nosocomial infection is increased (Mathur & Agarwal P.K. *et al*., 2010) [5].

Various antibiotics are available in the market to treat the bacterial infectious diseases by working on various targets to inhibit pathogen growth. Gram-positive and gram-negative bacteria can be inhibited by antibiotics viz. Chloramphenicol, Nalidixic acid, Rifampicin and ampicillin, either by blocking protein, DNA, RNAs or peptidoglycan synthesis respectively (Williamson & Salmond G.P *et al*., 2006) [6].

However, the development of bacterial resistance to antibiotics enforces the search for new antibacterial agents (Alanis*et al*. 2005). WHO emphasized research for natural components from herbal medicines to find new antibacterial agents (Mahady & G.B. *et al*, 2005) [9].

Currently available synthetic antioxidants like Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been

suspected to cause or prompt negative health effects. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow S.M *et al* 1990, Branen A.L *et al.*, 1975, Pourmorad F. &, Shahabimajd N *et al*., 2006) [10-12].

In the present work, some selected plants are screened for their potential antibacterial activities.

2. Necessity of work

- Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world.
- Increasing trends of multidrug resistance among emerging and reemerging bacterial pathogens to the available modern drugs or antibiotics.
- Plants are the potential source of natural antioxidants. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used antioxidants. Free radicals and other reactive species present in the body can be generated both endogenously and exogenously.
- These synthetic antioxidants also show low solubility and moderate antioxidant activity.

Objectives of the Study

1. Preliminary phytochemical analysis of dried leaf powder of selected plant species.
2. Quantitative phytochemical estimation of different extracts of the selected plant species.
3. Evaluation of antimicrobial activity of plant extracts against selected Gram-positive and Gram-negative bacteria *In-vitro*.

Expected Results

The outcomes from this study should be:

1. Providing information about the presence or absence of various plant metabolites that are responsible for their antimicrobial activity, this could be used in future for their isolation from crude leaf extracts.
2. Providing information on antibacterial activity of plants, to be used as basic pharmacological data for the clinical treatment in future.
3. Providing new sources of antimicrobial agents that can be used against microorganisms causing different types of infections.

3. Material & Methodology

Materials

Plant species: Collection and Authentication

The medicinal plant species *Putranjiva roxburghii* leaf was collected from local region, SMS campus near doctor's gharlos Jaipur (Gangwal Park) Rajasthan India, during 25 November 2017.

The medicinal plant species *Citrullus colocynthis* was collected from local region, piloting area Bhilwara Rajasthan India, during 10 December 2017.

The medicinal plant species *Achyranthes aspera* was collected from local region, Govt. Girls College Bhilwara Rajasthan India, during 25 January 2018.

The collected plants material was properly identified by Professor B.L Yadav department of Botany of Mewar University. Plant aerial part washed three times by running tap water, dried and powdered for further use.

Test organisms:

Microbial strains

Table: 3.1: Bacterial strains selected for antimicrobial study.

Gram (+) bacteria	Gram (-) bacteria
S. haemolyticus (+ve)	*K. pneumonia* (-ve)
B. cereus (+ve)	*E. faecalis* (+ve)

Methods

Preparation of extracts:

Fresh and mature leaves and aerial parts from selected medicinal plant species collected randomly from the local region of Rajasthan. Plant leaves and aerial parts were washed three times by running tap water, dried and powdered for further use.

Aqueous Extraction (Akueshi & Ngurukwem B *et al.*, 2002) [13]

- 25 gm of air dried powder was placed in hot distilled water and boiled for 30min.
- Kept undisturbed for24hr.
- Filtered off using sterile filter paper (Whatman no. 1) into a clean conical flask.
- Aqueous solvent was removed under pressure using a rotatory vacuum evaporator at 60 °C.
- The dried residue of crude extract was resuspended in 20%DMSO.
- And then stored in dark bottles at 4 °C.

***Solvent* Extraction (**Okemo & Fably W *et al.*, 2001) [14]

- Dried powdered leaves were extracted with solvent (95% (v/v) ethanol/acetone) using Soxhlet extractor.
- 25 gm powder was put in soxhlet thimble and into a soxhlet thimble tube.
- 250 ml of solvent (95% ethanol/acetone) was added to soxhlet flask then extracted at 40°C until the extract was clear or about 12hr.
- Solvent was removed under pressure using rotatory evaporator at 40 °C.
- The dried residue of crude extract was resuspended in 20% DMSO
- Stored in dark bottles at 4 °C.

Preliminary phytochemical analysis:

Qualitative phytochemical analysis of dried leaf powder of selected plants was done by using methods of (Harbone & J.B *et al*., 1998).

Phytochemical screening

The preliminary phytochemical screening was performed according to Harborne methods (Harbone & J.B., *et al*., 1998). The different crude extracts of leaves and stems of three important medicinal plants respectively *Putranjiva roxburghii, Achyranthus aspera* and *Citrullus colocynthis* were tested for the presence of flavonoids, terpenoids, alkaloids, saponins, proteins and sugars. The qualitative results are expressed as (+) for the presence and as (-) for the absence of a phytochemical type of compounds.

Phytochemical screening

Test for alkaloids (Sofowara A.1993) [16]

Mayer's Test: Test solution (1ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodine solution) were added into it and then cream color precipitate

was observed.

Dragendorff's test: To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added by the side of the test tube. A prominent red precipitate indicates test as positive (Sofowara A *et al*., 1993) [16].

Wagner's Test: To 2 ml extract was added a few drops Wager's reagent. Formation of reddish brown precipitate indicated the presence of alkaloids (Kokate C K and Gokhale SB *et al*., 2001).

Hager's Test: To 2 ml extract, were added a few drops of Hager's reagent (saturated solution of picric acid). Formation of yellow color precipitate signified positive result.

HCl Test: To 2 ml of extract, were added 1 ml of 1% HCl and heat gently. Then were added few drops of Mayer's reagent and wagner's reagent to the mixture. Turbidity of resulting precipitate was the evidence of the presence of alkaloids.

Tannic acid test: Alkaloids give buff color precipitate with 10% tannic acid (Software A.1993).

Test for tannins (Software A. 1993; Trease GE and Evans WC. 1989) [18].

Ferric Chloride test: To test solution added 10 ml distilled water, then filtered, in the filterate 2 ml FeCl3 (10%) was added, blue-black or green precipitate formed, indicate the presence of tannins.

Gelatin test: To the test solution added 1 ml of 1 % gelatin solution and 1 ml of 10% NaCl, white precipitate of gelatin indicate the presence of tannins.

Vanillin hydrochloride test: To the test solution added 1ml of vanillin hydrochloride solution; purple red color indicates the presence of tannins.

Test for cardiac glycosides (Trease GE and Evans WC 1989) [18]

Keller-Killiani test: To an extract, added 4 ml of glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid (2 ml) was added. Brown ring obtained at interface, indicate the presence of cardiac glycosides.

Salkowski test (Sofowara A. 1993) [16].

To the test solution added 2 ml of chloroform and 1 ml H2SO4, reddish brown color at interface, indicate the presence of cardiac glycosides.

Baljet test: (Kokate C.K, Purohit A P and Gokhale SB. (2008).

Solution-1: picric acid (1 g) in 100 ml of ethanol. Solution-2: NaOH (10 g) in 100 ml of water.

Mixed both solution. To the test solution added 2 to 3 drops of combined solution, orange to deep red color, indicate the presence of cardiac glycosides.

Test for steroids (Khandelwal KR. 2004)

Libermann test: To the test solution added 10 ml of chloroform then filtered. To the 2 ml filterate added 2 ml ofacetic anhydride and con. H2SO4. Blue green ring indicate the presence of steroids in thesample13.

Test for flavonoids (Khandelwal KR. 2004) [19]

Alkaline reagent test-

To the test solution added few drops of NaOH solution, formation of intense yellow color, which turns to colorless on the addition of few drops of diluted acid, indicates presence of flavonoids.

Lead acetate test: The extracts were treated with few drops of 10% lead acetate solution. The formation of precipitate confirmed the presence of flavonoids (Khandelwal KR. 2004) [19].

Test for terpenoids (Harborne JB. 2004)

Salkowski test: To the test solution added 2 ml of chloroform and 1 ml H2SO4, reddish brown color at interface, indicate the presence of terpenoids.

Test for proteins (Kokate CK *et al*., 2000) [21].

Ninhydrin test: To the test solution added 1 ml of 0.2% ninhydrin solution, violet color indicates the presence of protein in sample.

Test for reducing sugar (Trease GE and Evans WC. 1989) [18]

Fehling's test-

Filtrate (1ml) was boiled on water bath with 1ml each of Fehling solution A & Fehling solution B; a colored product indicates the presence of sugar.

Test for saponins (Software A.1993) [16].

Foam test: To the 0.5 ml of test solution added 2 ml distilled water and shake the all tubes, if foam produced persist for 10 min, indicate the presence of saponins.

Preparation of bacterial inoculum:

- Inoculum was prepared by picking loopful of isolated bacterial colonies from 24 hr old culture grown on nutrient agar and suspended in 5 ml of sterile nutrient broth.
- The broth culture was incubated at 37°C for 4hr.
- The resulting suspension was vortexed and turbidity was adjusted with nutrient broth to yield 1-2x108 – cfu/ml optically comparable to that of the 0.5 Mc Farland standard.

Antibacterial assay:

Screening of plants crude extract for antibacterial activity will be done by disc diffusion method (Bauer & Turck M *et al*., 1966) [22].

- Suspension of bacterial strain (4hr old) in nutrient broth was made.
- Turbidity was adjusted of 0.5 Mc Farland standards (108cfu/ml).
- Suspension was spread over the plate containing Mueller Hinton agar using sterile cotton swab.
- The disc 6 mm was saturated with the extract, and allowed to dry and was introduced on the upper layer of seeded agar plate.
- The plates were incubated overnight at 370C.
- Microbial growth was determined by measuring the diameter of zone of inhibition.
- For each bacterial strain, control was maintained where pure solvent (DMSO) was used instead of extract.
- The experiments were done in duplicates and mean values with standard error were presented.
- The results were compared with the standard antibiotics Chloramphenicol (100µg/ml/disc).

Determination of total phenolic

Total phenol contents in the extracts will be determined by modified Folin-Ciocalteu method. (Wolfe, & Liu R.H. *et al*., 2003) [23].

- An aliquot of the extract was mixed with 5 ml FC reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate.
- The tubes were vortexed for 15 sec and allowed to stand for 30 min at 400 C for color development.
- Absorbance was then measured at 765 nm using UV-Visible spectrometer.
- Samples of extract were evaluated at a final concentration of 0.1mg/ml.
- Total phenolic content were expressed as mg/g tannic acid equivalent using equation based on calibration curve

Determination of total flavonoids

- Total flavonoids will be estimated using the following method (Ordon, ez A.A.L., Gomez & Isla M.I *et al*., 2006) [24].
- To 0.5 ml of sample, 0.5 ml of 2% AlCl3 ethanol solution was added.
- After one hour at room temperature, the absorbance was measured at 420nm.
- A yellow color indicated the presence of flavonoids.
- Extract samples were evaluated at a final concentration of 0.1mg/ml.
- Total flavonoids were calculated as quercetin (mg/g) using the equation based on calibration curve.

Determination of MIC:

MIC was determined by the tube dilution method. (Baron and Fingold 1990) [25]. The MIC was taken as the least concentration that inhibited the growth of the test organisms (Baron J. E., & Fingold S. M. 1990) [25].

4. Results & Discussion

Table 4.1: Preliminary phytochemical analysis of screened medicinal plants *C.colocynthis*

Sr. no.	Tests	Reagents used	Aerial part
	Water extractives		
1	Strach	I2-KI	-ve
2	Tannins	Acidic FeCl3	+ve
3	Saponins	H2SO4 + Acetic anhydride	+ve
4	Proteins	Million's test	+ve
5	Reducing sugars	Benedict's test	+ve
6	Terpenoids	Salkowski's test	-ve
	Methanol extractives		
1	Alkaloids	Mayer's	+ve
		Wagner's	+ve
		Dragnendorff's	+ve
2	Flavonoids	Hcl+Mg turnings	+ve
3	Glycosides	Benzene + hot ethanol	+ve

+ ve: Present, -ve: Absent.

Table 4.2: Qualitative phytochemical screening of *Putranjiva roxburghii* leaf sample

Extract						
Phytochemical constituents		**Acetone**	**Aqueous**	**Chloroform**	**Ethanol**	**Methanol**
1	Test for Alkaloids					
	Dragendorff's	+	+	+	+	+
	Mayer's Test	+	–	–	+	+
	Wagner's Test	+	+	–	+	+
	HCl Test	+	–	–	+	+
2	Test for Carbohydrates					
	Molisch's Test	+	–	–	–	+
	Fehling Test	+	+	–	+	+
	Benedict's Test	–	–	+	–	–
3	Test for Flavanoids					
	Alkaline Test	+	+	–	+	+
	Conc. H_2SO_4 Test	+	+	+	+	+
	Pew's Test	+	–	–	+	–
	Lead acetate	+	+	–	+	+
4	Test for fixed oils					
	CuSO4 Test	+	+	–	+	+
5	Test for Phenols					
	Ferric chloride Test	+	+	+	+	+
	Potassium Dichromate Test	+	–	–	+	+
6	Test for Tannins					
	Ferric chloride Test	+	+	+	+	+
	Braymer's Test	+	+	+	+	+
7	Test for saponins					
	Foam Test	–	+	–	–	+
8	Test for Glycosides					

	Keller kiliani Test	+	–	+	+	+
	Glycoside Test	+	+	+	+	+
9	Test for Coumarins					
	10%NaOH Test	+	+	+	+	+
10	Test for Sterols					
	Salkowshi's Test	+	–	–	+	+
	Keller killiani Test	+	–	+	+	+
11	Test for Proteins					
	Biuret Test	–	–	–	–	–
	Xanthoproteic Test	+	+	+	+	+
	Conc.H_2SO_4Test	–	–	–	–	–
12	Test for Amino acids					
	Ninhydrin Test	+	–	–	–	–
13	Test for Terpenoids					
	Salkowshi's Test	+	–	+	+	+

+ Present, - Absent.

Table 4.3: Qualitative phytochemical screening of various extracts of *Achyranthes aspera* Aerial parts

Extracts								
Phytochemical constituents	**Tests**	**PEAA**	**BEAA**	**CEAA**	**EAEAA**	**EEAA**	**AEAA**	**MEAA**
1	Test for Alkaloids							
	Dragendroff's Test	–	+	+	+	+	+	+
	Tannic acid Test	–	–	+	–	+	+	+
	Wagner's reagent	–	–	+	–	+	+	+
	Mayer´s Test	–	+	+	+	+	+	+
2	Test for Tannins							
	Ferric Chloride Test	–	–	–	–	+	+	+
	Vanillin HCl Test	+	+	+	+	+	+	+
	Gelatin Test	+	+	+	+	+	+	+
3	Test for Carbohydrates							
	Molisch's Test	–	+	+	+	+	+	+
	Test for Reducing Sugar							
	Fehling′s Test	+	+	+	+	+	+	+
4	Test for Flavanoids							
	Alkaline Test	–	–	+	+	+	+	+
	Lead acetate	–	–	–	–	+	+	+
5	Test for Terpenoids							
	Salkowski Test	–	–	–	+	+	+	+
6	Test for Phenols							
	Ferric chloride Test	–	–	–	+	+	+	+
8	Test for saponins							
	Froth Test	–	–	+	+	+	+	+
9	Test for Glycosides							
	Keller kiliani Test	+	+	+	+	+	+	+
	Salkowski Test	–	–	–	+	+	+	+
	Baljet Test	+	+	+	–	+	+	+
10	Test for Sterols							
	Libermann-Buchard Test	+	+	+	–	+	+	+
11	Test for Proteins							
	Ninhydrin Test	–	–	–	–	–	–	+

(+) trace amount and (-) completely absent. Abbreviations: PEAA- Petroleum ether extract of *A. aspera*; BEAA- Benzene extract of *A.aspera;* CEAA- Chloroform extract of *A. aspera;* EAEAA- Ethyl acetate extract of *A. aspera*; EEAA- Ethanolic extract of *A. aspera;* AEAA- Aqueous extract of *A. aspera*.

Quantitative phytochemical analysis:

Table 4.4: Spectrophotometric determination of absorbance for chlorophyll a (Ch-a), chlorophyll b (Ch-b) and total carotenoids (C x+c) by acetone extractant solvent

Extractant Solvent	***Citrullus colocynthis***		
	A663nm Chl a	**A645nmChl b**	**A430nm C x+c**
Acetone	1.51	0.836	1.462

A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C x+c = Carotenoids

Table 4.5: Determination of total sugar content *Citrullus colocynthis*

OD	**Concentration**
0.268	0.1
0.362	0.2
0.481	0.4
0.609	0.6
0.761	0.8
0.877	1

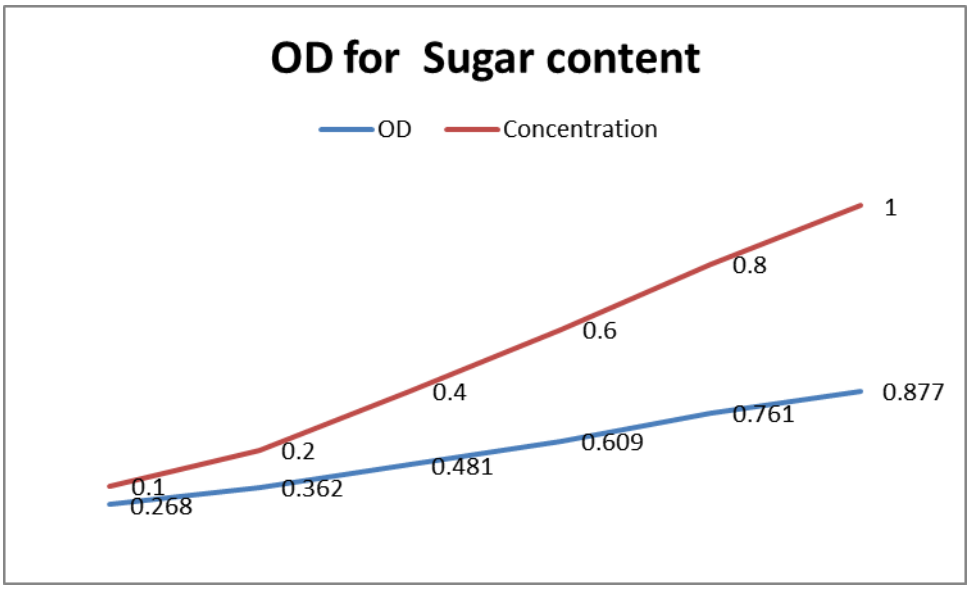

Fig: 4.1: Determination of sugar content using Anthrone a standard

Table 4.6: Total Alkaloids contents in *Citrullus colocynthis* plant sample

Sample number	Source (plant extracts)	UV–vis (mg/100 g)
1	Methanolic extract of C. colocynthis	73.213
2	n-Hexane faction of C. colocynthis	65.442
3	Chloroform faction of C. colocynthis	55.342
4	Ethyl acetate faction of C. colocynthis	53.982
5	n-Butanol faction of C. colocynthis	69.872
6	Aqueous faction of C. colocynthis	85.962

Determination of phenolic content using *mg gallic acid/g of dry material* as a standard

Table 4.7: Total Flavonoids contents in Citrullus colocynthis plant sample

Sample number	Source (plant extracts)	UV–vis (mg/100 g)
1	Methanolic extract of C. colocynthis	75.613
2	n-Hexane faction of C. colocynthis	25.542
3	Chloroform faction of C. colocynthis	52.182
4	Ethyl acetate faction of C. colocynthis	84.128
5	n-Butanol faction of C. colocynthis	65.772
6	Aqueous faction of C. colocynthis	63.652

Determination of flavonoid content using *mg* Catechin */g of dry material* as a standard

Table 4.8: Total Tannin contents in Citrullus colocynthis plant sample

Sample number	Source (plant extracts)	UV–vis (mg/100 g)
1	Methanolic extract of C. colocynthis	52.564
2	n-Hexane faction of C. colocynthis	25.763
3	Chloroform faction of C. colocynthis	62.831
4	Ethyl acetate faction of C. colocynthis	75.423
5	n-Butanol faction of C. colocynthis	70.712
6	Aqueous faction of C. colocynthis	69.342

Determination of Tannin content using *mg* tannic acid */g of dry material* as a standard

Table 4.9: Quantitative phytochemical analysis of *Putranjiva roxburghiiL* leaves extract

Sample/Standards	Total phenols
Putranjiva roxburghii	
Methanol	373.6 ± 1.4
Acetone	176.0 ± 1.3
Chloroform	150.0 ± 0.8
Ethanol	59.2 ± 0.6
Aqueous	36.9 ± 0.3
Hexane	-

mg GAE/g of extract; at 500 µg/ml; equivalent to FeSO4.7H2O (µM); absorbance at 695 nm; - = not calculated. *P. roxburghii*: in methanol

Table 4.10: Quantitative phytochemical analysis of aerial parts extract *Achyranthes aspera* L.

S.No	Secondary Metabolites	Result (mg/gm)
1	Phenol	172.2
2	Flavonoids	68.00
3	Tannin	25.3
4	Terpenoids	20.00

Table 4.11: The effect of methanol extract of C. colocynthis aerial part against the of standard organisms (-ve& +ve Bacteria)

	Concentration			
	Standard	Plant extract		
Standard bacteria	Chloramphenicol (100µg/ml)	1000 (µg/ml)	500 (µg/ml)	10 (µg/ml)
K. pneumonia (-ve)	25	19	16	8
S. haemolyticus (+ve)	20	10	9	8
B. cereus (+ve)	20	15	11	9
E. faecalis (+ve)	19	17	10	8

*Values are mean inhibition zone (mm)

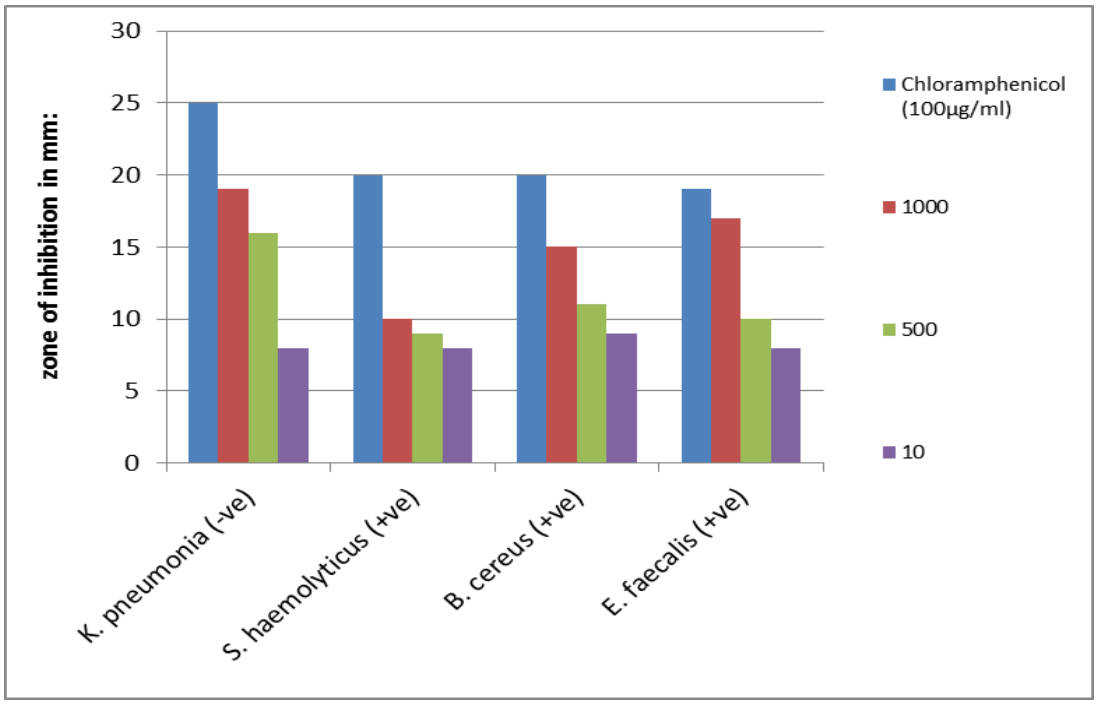

Fig 4.2: The effect of methanol extract of C. colocynthis aerial part against the of standard organisms (-ve & +ve Bacteria)

Table 4.12: The effect of methanol extract of Achyranthus aspera aerial part against the of standard organisms (-ve& +ve Bacteria)

Concentration				
	Standard	Plant extract		
Standard bacteria	Chloramphenicol (100µg/ml)	1000 (µg/ml)	500 (µg/ml)	10 (µg/ml)
K. pneumonia (-ve)	22	17	12	9
S. haemolyticus (+ve)	20	10	8	8
B. cereus (+ve)	20	16	15	9
E. faecalis (+ve)	20	15	12	8

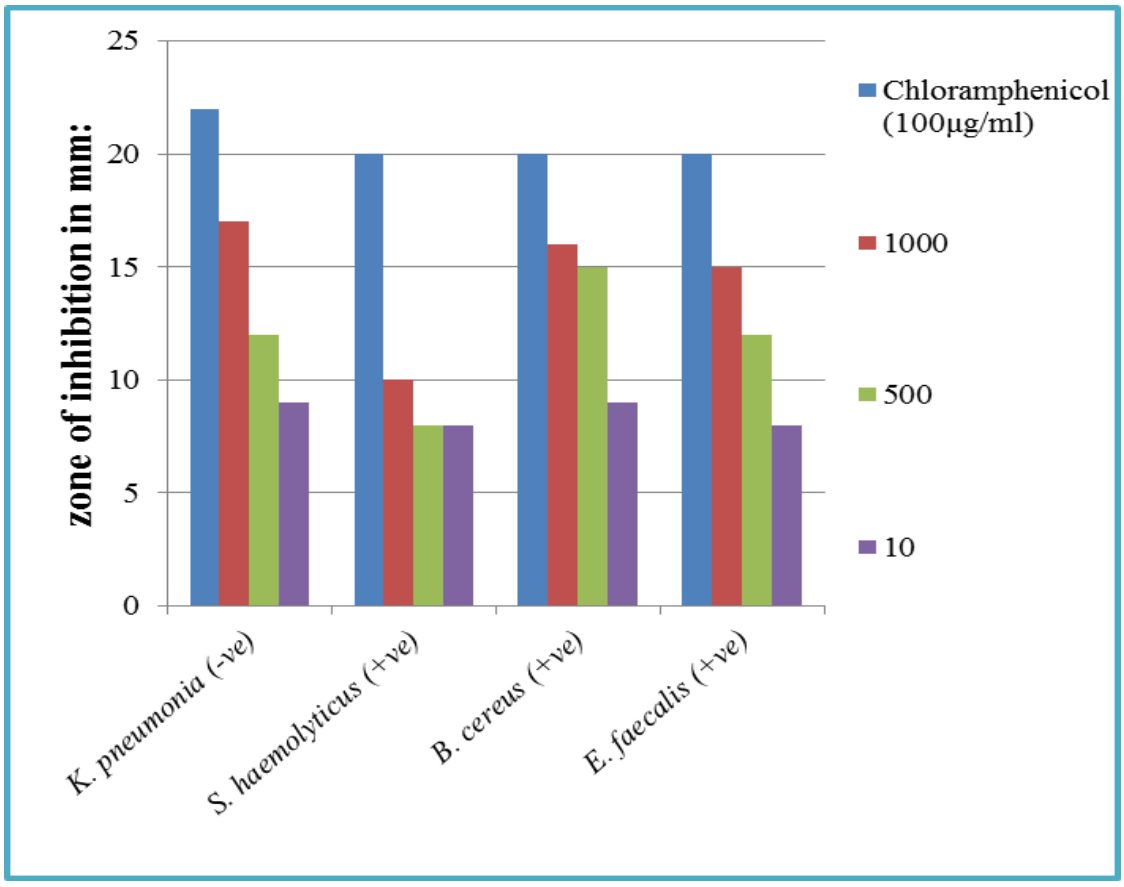

Fig 4.3: The effect of methanol extract of Achyranthes aspera aerial part against the of standard organisms (-ve& +ve Bacteria)

Table 4.13: Antibacterial assays of *Putranjiva roxburghii* leaf

Table 2: The effect of methanol extract of Putranjiva roxburghii leaves against the of Standard Organisms (-ve & +ve Bacteria)

	Concentration			
	Standard	Plant extract		
Standard bacteria	Chloramphenicol (100µg/ml)	1000 (µg/ml)	500 (µg/ml)	10 (µg/ml)
K. pneumonia (-ve)	22	17	14	9
S. haemolyticus (+ve)	20	16	12	8
B. cereus (+ve)	25	18	14	8
E. faecalis (+ve)	20	16	13	9

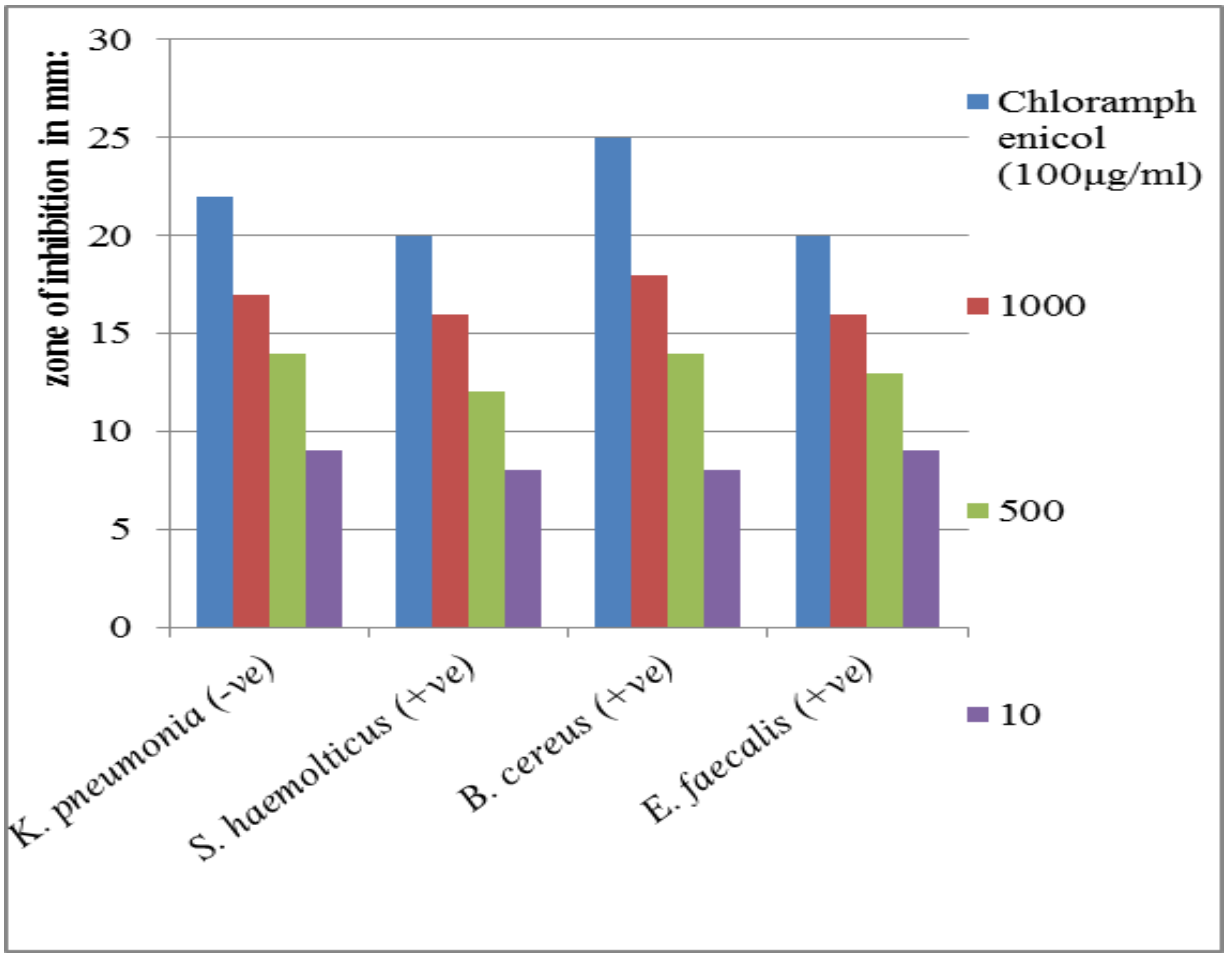

Fig 4.4: The effect of methanol extract of Putranjiva roxburghii leaves against the of standard organisms (-ve & +ve Bacteria)

Preliminary phytochemical analysis of *Citrullus colocynthis, Putranjiva roxburghii* and *Achyranthus aspera* indicates the presence of tannins and terpenoids. However, *Putranjiva roxburghii,* and *Citrullus colocynthis* shows absence of terpenoids in aqueous extract (table 4.1 & 4.2) while terpenoids were present only in aqueous extract except all the extracts of aerial part *of A. aspera*. For Alkaloids, respectively three plants extract shows positive Wagner's, Mayor's and Dragendorff's test (table 4.1, 2, 3).

Phytochemical tests were for *Citrullus colocynthis* carried out of water extractives for starch, tannins, saponins, proteins, and reducing sugars and on methanol extract for alkaloids, glycosides and flavanoids. The detailed of phytochemical screening in the two forms of extract is given in table 4.1. Phytochemical screening portrays that most of the natural products tested for were present in the plant material except starch which were not detected in any of the tested fractions. Analysis of saponins, proteins, reducing sugars, alkaloids, glycosides and flavonoids in the aerial part extracts was positive. Aerial part of *C. colocynthis* extract showed positive results for tannins while the aqueous extract showed negative results for starch and terpenoids.

The results of qualitative screening of phytochemicals of *P. roxburghii* leaves showed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Phenols, Tannins, Fixed oils, Coumarins, Sponins, Sterols and Terpenoids (Table 4.2). While aqueous extract given the absence of alkaloids, terpenoids, amino acids, sterols. On the other hand this plant showed some properties against bacteria. Highest antibacterial activity was seen in methanolic extract is given (Table 4.11, Fig. 4.2).

The phytochemical characteristics of the sequential extracts of *A. aspera* aerial part were investigated and are summarized in table (Table 4.3). Different extracts of aerial part of *A. aspera* showed the presence of tannins, alkaloids, cardiac glycosides, reducing sugars and saponin. It was seen that alkaloids,

tannins, cardiac glycosides, reducing sugar and saponins were present in all extracts while proteins was absent in respectively all extracts except to methanolic extract. This indicates that the presence of secondary metabolites may have suppressed the activity of protein. In addition to this, the solvents might have also denatured the protein. Steroids were present in all extracts except to chloroform, ethanol and ethyl acetate extract, whereas flavonoids and terpenoids were present in all extracts except benzene extract. The results confirm the presence of constituents which are known to exhibit medicinal properties.

Preliminary phytochemical screening of the various extracts of *Achyranthes aspera* leaves revealed the presence of cardiac glycosides, reducing sugars, alkaloids, and tannins were the most prominent. Terpenoids, reducing sugars, cardiac glycosides were prominently found in sequential extracts of aerial part. These compounds may be responsible for antioxidant activity and antimicrobial activity and may serve as a substitute for synthetic drugs (Beulah S, Reddy U2011).

For aerial part, flavonoids and terpenoids were found. Flavonoids were present in ethanolic chloroform, methanolic, ethyl acetate extract and aqueous sequential extracts of aerial part and terpenoids were found to be present in ethanolic, chloroform, methanolic, ethyl acetate extract and aqueous extracts while terpenoids were absence in all the extracts except benzene extract. Steroids were also present in petroleum ether and chloroform extract. The results of phytochemical screening of leaves are tabularized in table 4.3. For various extracts of aerial part, alkaloids were found in benzene, chloroform, ethyl acetate, ethanolic and aqueous extracts of aerial part of *Achyranthes aspera*. Tannins were found to be present in almost every extract Vanillin-HCl test. Ninhydrin Test for proteins were absence in all the extracts except methanolic extract. Cardiac-glycosides were found to be present in all extract Keller kiliani Test while in other tests Salkowski Test were present in ethanolic, methanolic, ethyl acetate extract and aqueous extract absence in other extracts Petroleum ether, Benzene and Chloroform extract. Steroids were present in all the extracts except benzene extract. Froth test for saponins screened their presence in chloroform, ethyl acetate, ethanolic, methanolic, and aqueous extracts of aerial part while absence in other extracts Petroleum ether, Benzene extracts of aerial part of *Achyranthes aspera*. The results of phytochemical screening for stems are tabularized in table 4.3.

All these phytochemicals are reported to possess various pharmacological actions and anti-oxidant properties. A water soluble alkaloid, Achyranthes was isolated from the plant which is reported (Neogi NC *et al*., 1970) [27] to possess cardiovascular activities and broncho-protective activities. The presence of flavonoids in the extract is responsible for the free radical scavenging effect observed. Plant phenolics like flavonoids and tannins act as primary antioxidants or free radical scavengers.

The estimation of polyphenols was confirmed through phenol reduction UV–vis spectroscopic method of phosphotungstic phosphomolybdic acids (Folin–Ciocalteu's reagent).

The results of the were further confirmed through UV–vis spectroscopy by using the Folin–Ciocalteu and tannic acid colorimetric method summarized in Table 4.6, 4.7that the aqueous extract of the aerial parts alkaloids 85.962 (mg/100 g) have the higher total phenolic contents. The highest contents of flavonoid were observed in the ethyl acetate extract of the aerial parts of C. colocynthis 84.128 (mg/100 g) given in table 4.7.

While the highest contents of tannin were observed in the ethyl acetate extract of the aerial parts of C. colocynthis 75.423 (mg/100 g) given in table 4.8.

The amount of total phenolic contents expressed as mg GAE/g of extract varied widely among plant extracts and ranged from 36.9 ± 0.3 to 373.6 ± 1.4 mg GAE/g of extract in *P. roxburghii*, (Table 4.8). Remarkable results were obtained with acetone extracts of all the tested plants in the decreasing order PRM > PRA > PRC> PRE >PRAq>PRH.

The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the extract of aerial parts of *A. aspera*.

Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. Phenolic compounds also possess anti-mutagenic and anti-tumor activities (Othman *et al*., 2007) [28]. In the current work, the phenolic content of the *A. aspera* extracts was measured (Table 4.10). The methanol extract of *A. aspera* showed higher amount of phenolic compounds compared to aqueous extract. The concentration of the phenolic compounds was increased with increase in the dose. The results are described as Gallic acid equivalents (GAE).

The presence of glycosides was detected in both parts (root and inflorescences) of *A. aspera*. Glycosides have been known to regulate blood pressure, although some workers have attributed the cardiac action of these oils due to the presence of alkaloids (Watt FM *et al*., 1984) [29].

Phytochemical research based on ethanopharmacological informations is generally considered an effective approach to the discovery of ant infective agents from higher plants (Kloucek *et al*., 2005) [30].

The presence of zones of inhibition on the seeded agar plates showed that the plant extract possesses antibacterial activity on the tested organisms which included both Gram positive and Gram negative organisms. Although the zones of inhibition were lower than that exhibited by the standard drug Chloramphenicol this could be due to the fact that the plant extract is crude and contains other constituents that do not possess antibacterial property. Also the ability of the extract to diffuse through the gel may be hindered because of large molecules (stearic hindrance). At higher concentrations of the extract, the zones of inhibition with the standard drug were comparable.

Methanol extracts of three important medicinal plants respectively *Putranjiva roxburghii, Achyranthus aspera* and *Citrullus colocynthis* showed activity against all the selected bacterial strains. Methanol extracts of *Achyranthus aspera* and *Citrullus colocynthis* showed min. zone of inhibition against the selected gram positive *S. Haemolyticus* (+ve) and maxi zone of Inhibition for gram negative bacteria *K. pneumonia* (-ve) (table 4.11 & 4.12, fig. 4.2 & 4.3).

Methanol extracts *Putranjiva roxburghii* of showed min. zone of inhibition against the selected gram positive *S. Haemolyticus* (+ve) and maxi. Zone of Inhibition for gram positive bacteria B. cereus (+ve) (table 4.13, fig. 4.4). Methanol extracts of three important medicinal plants respectively *Putranjiva roxburghii, Achyranthus aspera* and *Citrullus colocynthis* showed min. zone of inhibition against the selected gram positive *S. Haemolyticus* (+ve). On comparing MIC concentration of different plant extracts with Standard Chloramphenicol (100µg/ml), it was observed that

methanol extracts of *Achyranthus aspera* and *Citrullus colocynthis* gave best result against K. pneumonia (-ve), whereas Methanol extracts *Putranjiva roxburghii* of showed min. zone of inhibition against the selected gram positive *S. Haemolyticus* (+ve) and maxi zone of Inhibition for gram positive bacteria *B. cereus* (+ve). In case of gram negative bacteria, the methanol extracts of *Achyranthus aspera* and *Citrullus colocynthis* worked best against both *K. pneumonia* (-ve), while Methanol extracts *Putranjiva roxburghii* of showed min. zone of inhibition against the selected gram positive *S. Haemolyticus* (+ve) and maxi zone of Inhibition for gram positive bacteria *B. cereus* (+ve) (table 4.13, fig. 4.4).

Generally, the antibacterial activity of the methanolic extract of *Putranjiva roxburghii* Wall against *Klebsiella pneumonia* and *Bacillus cereus*, agrees with earlier works by (Minj *et al*., 2016) [31].

The present study was designed to obtain preliminary information on the antibacterial activity of 12 methanolic plant leaf extracts. Disc diffusion method was used in this study. Out of 12 extracts tested only methanolic extracts of Putranjiva roxburghii Wall and Artabotrys hexa petalus exhibited good antibacterial activity and gave zone of inhibition followed by the methanolic extract of Aegle marmelos (L.) Correa, *Anethum graveolens*L and *Eupatorium capillifolium* (Lam.) Small against *Erwinia herbicola* and *Salmonella typhi*. (Eloff. JN *et al*., 1998) [33] found that methanol was the most effective solvent for plant extraction than any other solvents. (Soniya *et al*., 2013) [34] also found methanol as the most effective solvent. *Putranjiva roxburghii* Wall showed highest inhibitory activity against bacterial pathogen. (Shahwar *et al*., 2012 [35] reported that extracts of *P. roxburghii* contains antioxidant activities. The present investigation also revealed antioxidant potential.

The preliminary qualitative phytochemical screening is reported in this paper. *C. colocynthis* found to contain phytochemicals namely, saponins, tannins, alkaloids, glycosides and flavanoids. The antimicrobial study by agar disc diffusion method shows that the plant has an antimicrobial activity comparable to that of commercial antibiotic Chloramphenicol. The antimicrobial property is claimed to be conferred by phytochemicals present in the plant. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo *et al.*, 1991) [36]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al.*, 1998) [37]. Flavonoids display a remarkable array of biochemical and pharmacological actions viz. anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. Flavonoides are also shown to inhibit microbes which are resistant to antibiotics by (Linuma *et al.* (1994) [38]. It was also found that alkaloids were present in the ethanolic extracts. It will be advisable to extract the leaf of *C. colocynthis* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for this purposes (Trease and Evans, 1982; Zee-cheng, 1997) [39, 40]. Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973) [41]. It has also been shown that saponins are active antifungal agents (Sadipo *et al.*, 1991) [36]. Herbal medicine represents one of the most important fields of traditional medicine all over the world (Hamil *et al.*, 2003) [42]. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (Cragg *et al.*, 1997) [43]. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Farnsworth and Morris, 1976; Service, 1995) [44, 45].

Results obtained for *C. colocynthis* revealed the presence of flavonoids, saponins, cardiac glycosides, sterols, terpenoids as stated in previous study(Hussain AI, Rathore HA, Sattar MZ, Chatha SA, Sarker SD, Gilani AH 2014; Prasad MP 2014 [47]; Elgerwi AA, Benzekri Z, El-Magdoub A, El-Mahmoudy A. 2013) [48].

While the *C. colocynthis* methanol extract showed maximum relative percentage inhibition as compared to plant extracts. Methanol leaf extracts exhibit maximum (84.9%) percentage inhibition against *S. aureus* followed by *B. subtilis* (83.2%), *K. pneumonia* (79.5%) and *P. aeruginosa* (73.9%) (Naz R, & Farooq U *et al*., 2011) [49].

The frequent emergence of antibiotic-resistant strains urges continual demand for new antibiotics. In many developing countries around 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for these processes, which synthesize pure chemical derivatives (Penso G., 1980) [50].

5. Summary & Conclusion

Preliminary phytochemical analysis revealed the presence of tannins and terpenoids. The other secondary metabolites like cardiac glycosides flavonoids, steroids, saponins, etc. were present in trace amounts in some of the plants which could be correlated to antimicrobial properties.

The exact nature and mode of action of these active constituents is quite obscure at this stage. Further work may, however, reveal whether these components act as intracellular bacterial enzyme inhibitor, or impair the cell wall synthesizing system of the cell, or any other biological reaction impairment which causes cessation of growth or death of bacterial cells. Thus, here, it is also not possible whether; these extracts are bactericidal or bacteriostatic in nature.

The antimicrobial activities of these plants for the treatment of diseases as claimed by traditional healers also need to be investigated. It may also help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of infectious diseases.

Chsapter-VI

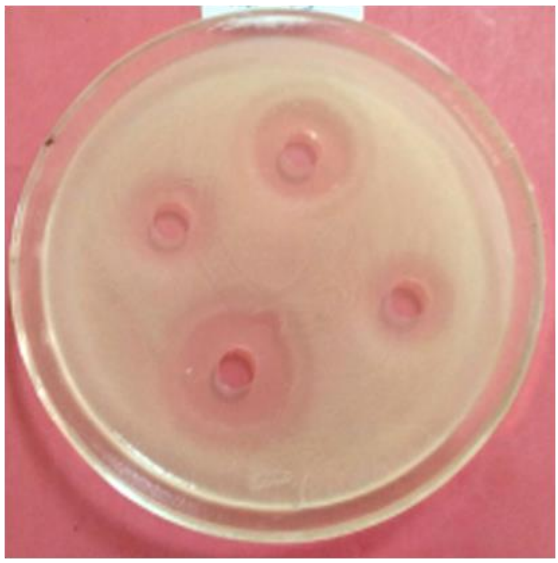

Plate 1

Methanol extract of *A. aspera* against *B.cereus*

Plate 2

Methanol extract of *A. aspera* against *S.heamolyticus*

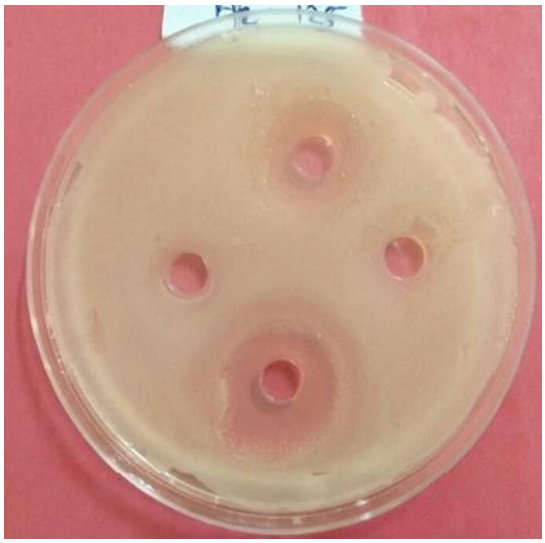

Plate 3

Methanol extract of *A. aspera* against *B.cereus*

Plate 4

Methanol extract of *Putranjiva* against *S.heamolyticus*

Plate 5

Methanol extract of *Putranjiva* against B. cereus

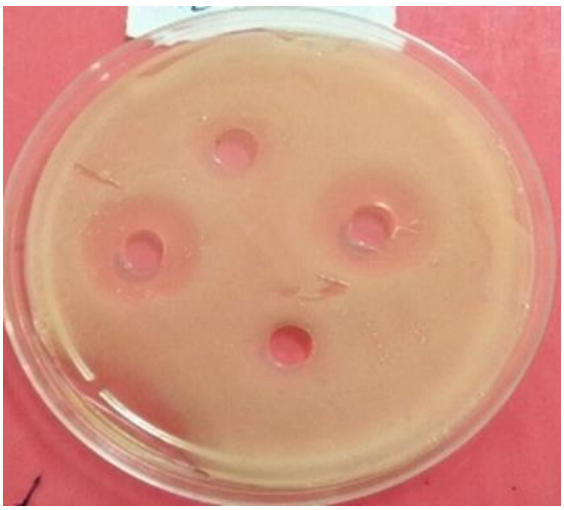

Plate 6

Methanol extract of *Citrullus colocynthis* against *S. heamolyticus*

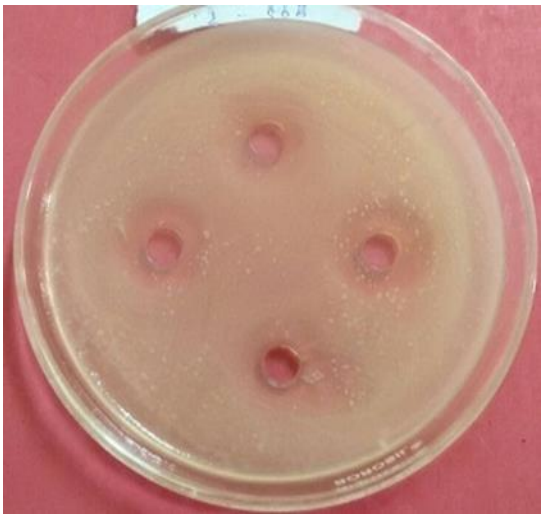

Plate 7

Methanol extract of *Putranjiva* against *S. heamolyticus*

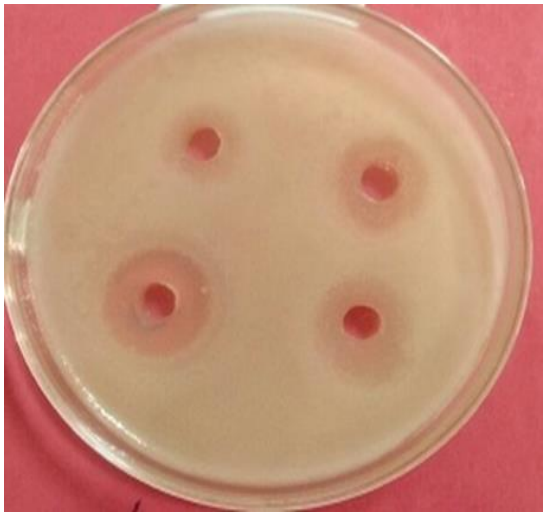

Plate8

Methanol extract of *Citrullus colocynthis* against *B. cereu*

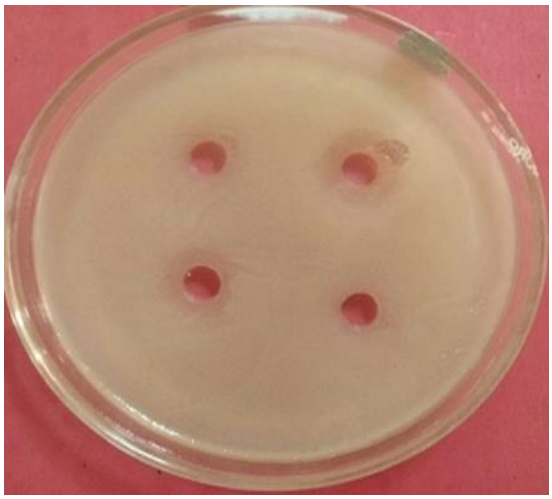

Plate 9

Methanol extract of *A. aspera* against *B. cereus*

Plate10

Methanol extract of *Putranjiva* against K.p

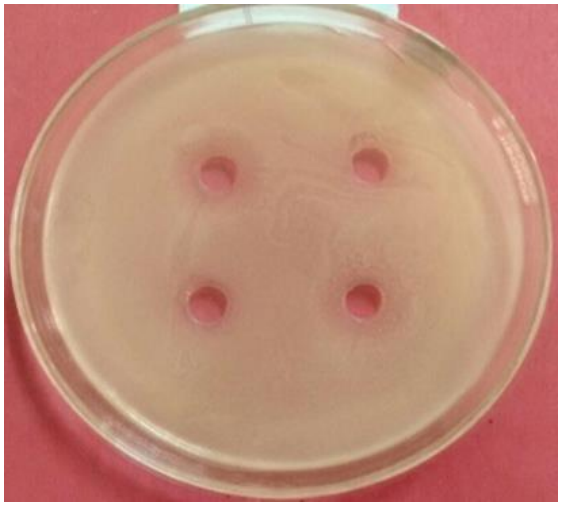

Plate 11

Methanol extract of *Putranjiva* against B. cereus

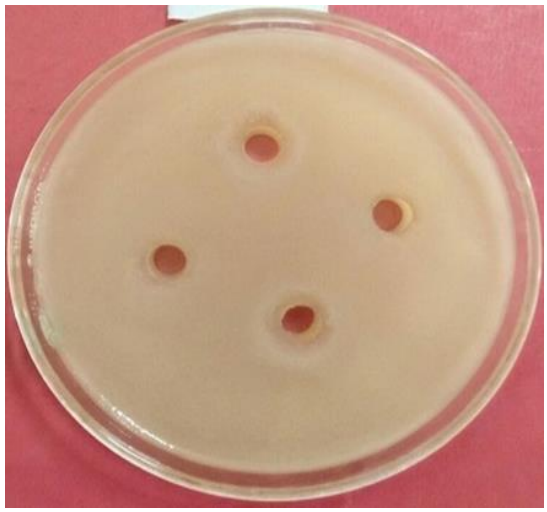

Plate12

Methanol extract of *Citrullus colocynthis* against *B.cereus*

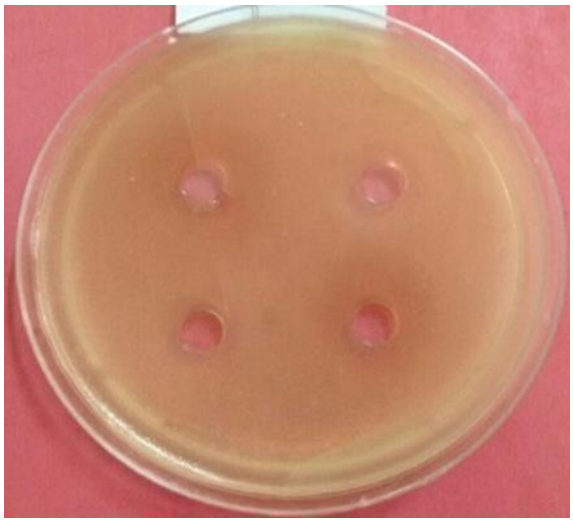

Plate 13

Methanol extract of *A. aspera* against *E.f*

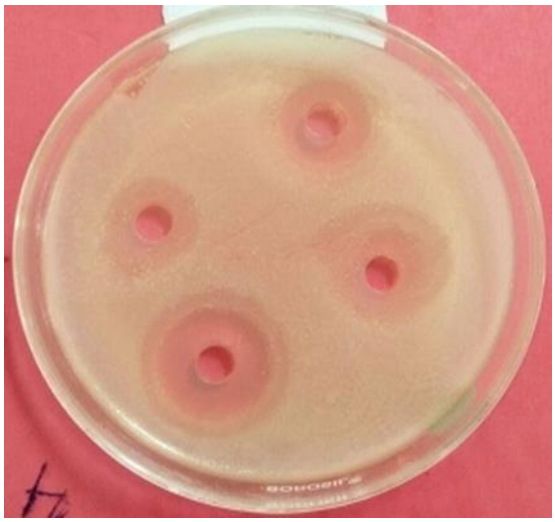

Plate14

Methanol extract of *Citrullus colocynthis* against *K.p*

Plate 15

Methanol extract of Citrullus colocynthis against B.cereus

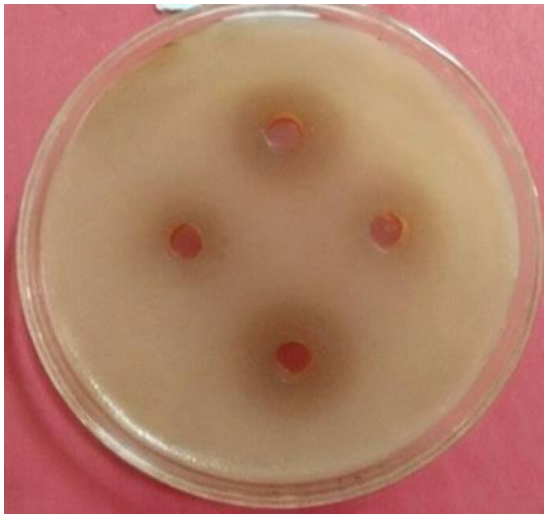

Plate16

Methanol extract of Putranjiva against E. f

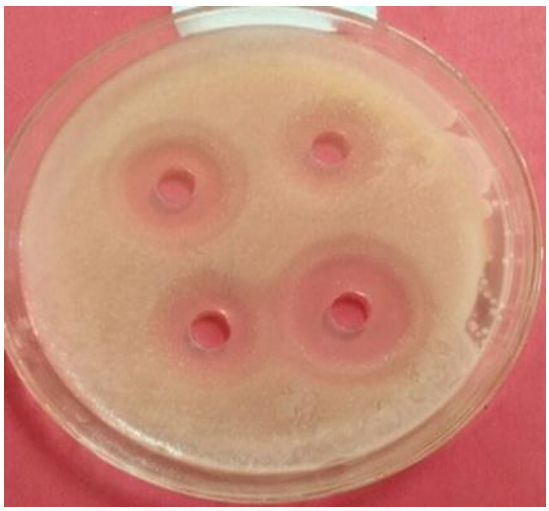

Plate 17

Methanol extract of A. aspera against K.p

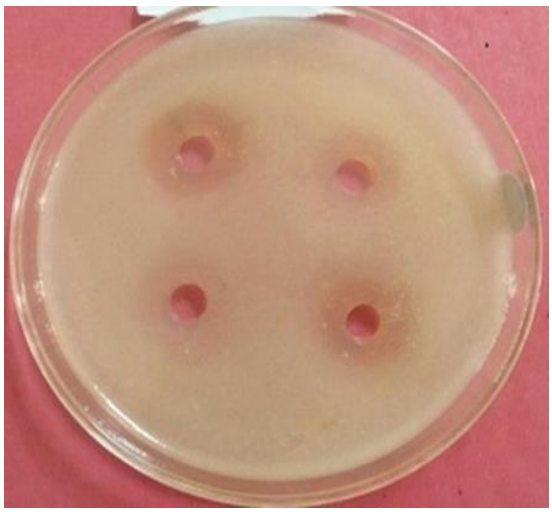

Plate 18

Methanol extract of Citrullus colocynthis against S.h

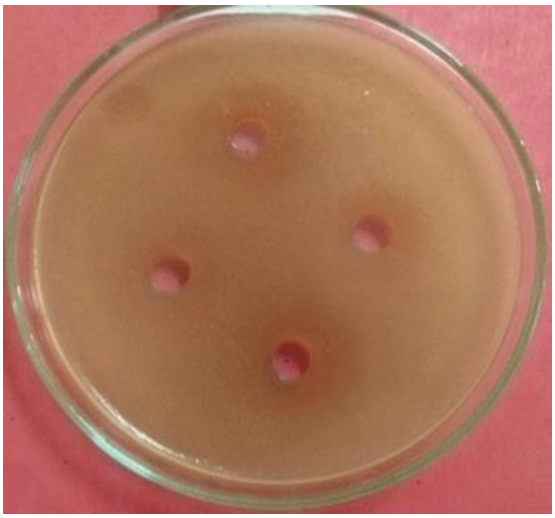

Plate 19

Methanol extract of Citrullus colocynthis against E.f

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