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Research Article

Screening of BADH Activity of *Borreria articularies* (Linn.) for the Inhibition of *P. aeruginosa*

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Abstract

Purposes: The present study was designed to investigate the antibacterial activities of the Ethanol and methanol extracts of the leaves of the plant *Borreria articularies (Linn.)* effects on microbial growth inhibition in vitro, microbial cells *in vivo* and molecular enzyme (BADH) targets *in vitro*.

Methods: The preliminary phytochemicals of the extracts was determined by the standard methods and aliquoted with Thin Layer Chromatography (TLC) and stored at 2-4^{o}C. fluorescein diacetate (FDA) and ethidium bromide (EB) live-dead cell viability test for distinguishing the membrane active phytochemicals of the plant extract. Betaine aldehyde dehydrogenase (BADH) activity was assessed by spectrophotometer. Alkaloids, glycosides, steroids, gums, saponin and reducing sugar were found in extracts.

Results: The results of the disc diffusion indicated that the crude extracts were able to inhibit the growth of bacteria within a concentration range of 0.5 to 2.0 mg/mL. At a similar concentration range (0.5 to 2.0 mg/mL) the extract inhibited the growth of 90.12**%** of the tested microorganisms. Bacterial cell viability was found minor in the phytochemicals of crude extract. Also, constituents of crude extract inhibited the BADH activity to protect the adaptation in stress environment of the bacteria.

Conclusion: Results of the present study showed the possible use of the studied plants extracts in the control of bacterial infections.

Keywords: Antibacterial activities, *Borreria articularies Linn.*, Methanol, Ethanol, Extract, Phytochemicals, BADH, Osmoprotectant, Live-dead cell

1. Introduction

Borreria articularis Linn. is a creeping branched annual evergreen herb in South East Asia. It is useful in treating fever, bladder stones, sores, wounds, headache, constipation, dental problems, fevers, diarrhea, head cold, hemorrhoids and conjunctivitis, gallstones, vitiated vata, pitta, arthritis, pain, muscle ache, edema, trauma, indigestion, colic, skin disease, menorrhagia and leucorrhea[1,2,3]. Plant principally contains alkaloids, glycosides, sterols, D-mannitol and ursolic acid. The plant contains sitosterol, ursolic acid and d-mannitol. Seeds contain isorhamnetin[4]. The chloroform extract of the aerial parts and roots of *Borreria articularis* contains triterpene, 3-α acetoxy-oleana-12-en-29-oic acid along with β-amyrin.

Microorganisms are major health concern although the current development of vaccination and chemotherapy. To identify a new antibacterial agents, it is not sufficient to study microbial growth inhibition but also important to study mode of action in pathogenic microorganisms such as effects on bacterial cell membranes and metabolic enzymes. Thus, plant compounds need to test after positive screening for microbial growth inhibition. Microorganisms can be inhibited by many possible target enzymes. The gram negative bacterium *Pseudomonas aeruginosa* is an important pathogen of plants and animals. Betaine aldehyde dehydrogenase (BADH) is a target enzyme for inhibition of *P. aeruginosa* growth. BADH catalyzes the synthesis of glycine betaine to inhibit *P. aeruginosa* in osmotic stress presented in the infected human tissues[5]. Glycine betaine may also protect intracellular enzymes and organelles against the adverse effects of increases in intracellular ionic strength or temperature[6,7,8].

Alkaloids are heterocyclic nitrogen compounds characterized by different antimicrobial activities. Plant-derived compounds are mostly secondary plant metabolites traditionally used for medicinal purposes. Activity the medicinal plants are varied due to species, topography and climate and categories of active principles[9,10] chemical compositions and its proportion in the crude extract modifies their antimicrobial activity. Some main categories of phytochemicals extracted from medicinal plants are examined to evaluate their antibacterial cellular activity. In this research work we investigated the scientific basis of the traditional uses of the plant for infection.

2. Material and Methods

2.1 Materials

2.1.1 Plant: *Borreria articularis* (Linn.), Bengali name Bonkodu, was collected from Gonoshasthaya kendra Medicinal Plant Garden, Bangladesh. The authenticity of the plant specimen has been confirmed by Bangladesh National Herbarium, Mirpur, Dhaka, Accession number: 32577.

2.1.2 Chemicals and biochemicals: Methanol and Ethanol were bought from Merck, Germany. Mayer's reagent, Dragendroff's reagent, Fehling's solution A, Fehling's solution B, Benedicts reagent, and Molisch reagent were prepeared in the laboratory. fluorescein diacetate (FDA) and ethidium bromide (EB) were purchased from Sigma, USA. Hanks Balanced Salt Solution (HBSS) was bought from Gibco, USA. Dubos Liquid Medium (DLM) and Dubos Agar Medium (DAM) were provided by Difco, USA. Betaine aldehyde chloride, glycine betaine (free base), choline chloride, NAD(P)1, NADPH, dithiothreitol (DTT), Tris were obtained from Sigma, USA. EDTA and glycerol were from Merck KGaA, Germany.

Ampholines and nitrocellulose membranes were from Bio-Rad (Hercules, Calif.), and Immobilon-PSQ membrane was from Millipore (Bedford, Mass.). 3-Dimethylsulfoniopropionaldehyde was collected from USA. g-Aminobutyraldehyde (diethylacetal form) was from Aldrich. g-Aminobutyraldehyde chloride was freshly prepared from the diethylacetal form by the procedure described by Flores and Filner[11]. Materials for column chromatography were purchased from Shimadzu, Japan. All other chemicals were from standard suppliers in analytical grade.

2.1.3 Organism: *Staphylococcus aureus* and *Candida albicans* were used as Gram-positive and Escherichia coli was used as Gram-negative microorganism. Nutrient Agar (NA) Medium (pH, 7.4 ± 0.2) and Sabeuraud Dextrose Agar Medium (SAD, pH at 25oC, 5.6 ± 0.2) were prepared in the laboratory for culture preparation. *Mycobacterium smegmatis* ATCC 607 and P. aeruginosa PAO1 were collected from ICDDR'B.

2.2 Method

2.2.1 Plant Extraction: The plant samples were primarily dried in the shadow and finally dried in an oven at 40°C temperature. The dried samples were ground to coarse powder with a mechanical grinder and then stored in a plastic container. 500g of the grounded plant was extracted by maceration with methanol and ethanol for 3 days at room temperature. The extracts were then filtered and concentrated by Rota-vapor at 40°C to give the crude extracts. All extracts were kept at 4°C until further investigations.

2.2.2 Preliminary phytochemical screening: The secondary metabolites such as alkaloids, Glycosides, Steroids, gums, reducing sugars, Tannins, Flavonoids, Saponins were screened using 10% (w/v) according to the common phytochemical methods described by Harbone[12].

2.2.3 Separation of phytochemical: The biologically active phytoconstituents were separated from the crude extracts by the column Chromatography and Thin layer chromatography. The column was packed with silica gel (Merk- 60, mesh 120). Slurry of silica gel in n-hexane was added into a glass column with the length and diameter of 55 cm and 1.1 cm respectively. When sufficient height of the adsorbent bed was obtained, a few hundred milliliter of n-hexane was run through the column for proper packing of the column. The sample was prepared by adsorbing 750 mg of n-hexane soluble fraction onto silica gel (Merk 60, mesh 120), allowed to dry and subsequently applied on top of the adsorbent layer. The column was eluted with n-hexane, ethyl acetate and methanol at different ratio. Solvent systems were used as mobile phases. Total 20 fractions each of 10 ml were collected. The column was eluted gradually with order of polarity of solvents from non polar to high polar. The individual eluents were examined by TLC.

Standard size TLC plates were cut into small pieces as Length – 7 cm, Width – 2.5 cm, Labeling space – 1 cm from both upper & lower side. The plant extract was used for chromatographic separation by TLC method. TLC plates in Iodine chamber showed a significant results in 9:1, 8:2, 7:3 and 6:4 mobile phase solvent ratio (n-hexane : ethyl acetate) and found spots in TLC with R_f values 0.63, 0.68, 0.66, 0.65 and indicated different verities of compounds (Figure 1).

Figure 1: TLC plate after placing in Iodine Chamber, Extract – methanolic, Solvent system –n-hexane : ethylacetate (8 : 2), R_f Value - 0.63, 0.68, 0.66, 0.65

2.2.4 Disc Diffusion antimicrobial Screening: Nutrient Agar (NA) Media (plant and tube) was prepared in Lab. The pH of the media was adjusted using NaOH/ HCl solution. The dissolved material was transferred to larger conical flask plugged by cotton and aluminum foil. The flask was autoclaved for sterilization at 121°C for 15min. After autoclaving the media was cooled about 45°C and it was poured on Petridis (20ml per plate) and in test tube for marking solid plate and slant medium. The Sabeuraud Dextrose Agar Medium (SAD) medium was prepared with same process. Test cultures were prepared from pure culture, by transferring the organism to sterile NA and SDA media with the help of a sterile inoculation loop inside laminar airflow chamber in aseptic condition. The inoculated mediums were then incubated at 37°C for 24hrs for growth of the organisms. All of the petridishes were sterilized at 180°C for 24 hrs. NA and SDA medium were taken into the petridishes. The plate was placed in the refrigerator for solidification. NA media were taken by sterile loop. Organisms were spread over the plate. Sterilized Pasteur pipette were taken and three holes were made. The plates were marked properly by name of the organism in one side and marking the position of the sample and standard disk on the other side. About 100µl of prepared sample solution was poured into each of the plate thru hole. The plates were then carefully stored in incubator for the development of the zone. Test samples were prepared by dissolving 50 mg of the ethanol and methanol extracts in 2 ml of respective solvents to make 1 mg/disc. All the plates (sample, standard and control) were incubated at a temperature of 25±2°C for 5 days. The radial growth of fungal colony was measured with a transparent scale in mm and the percentage of inhibition of mycelial growth was calculated using the following equation:

$$I = \frac{C - T}{C} \times 100$$

Here, I = Percentage of inhibition, C = Diameter of fungal colony in control, T = Diameter of fungal colony in treatment

2.2.5 Cell Viability antimicrobial Screening: Mycobacterium smegmatis ATCC 607 was inoculated on Lowenstein-Jensen Medium and incubated at 37°C for 72 hr. The stock slants were stored at 4°C for later use. Dubos Liquid Medium (DLM) with 0.4% glycerol was used for broth cultures after inoculation with the appropriate organism from a stock culture and incubation at 37°C on a rotary shaker at 180rpm. Dubos Agar Medium (DAM) was used for plating using same process of DLM except for the addition of 1.5% agar. Single-cell suspensions were prepared to ensure reliable plate and total counts to prevent the clump of organism in liquid medium for their lipophilic nature to the cell walls. DLM culture (2.0 ml aliquot) was centrifuged at room temperature for 15 min. Hanks Balanced Salt Solution (HBSS) with 0.05%Tween-80 was used for decantation of supernatant fluid and resuspension of cell pellet. Single-cell suspension containing 10^6 to 10^7 cells per milliliter was filtered through a 5 µm membrane filter using 10 ml syringe. All viability tests were performed using single-cell suspensions.

The fluorescein diacetate (FDA) and ethidium bromide (EB) fluorescent viability test was used to find living and dead cells. A stock solution of FDA (Sigma, U.S.A.) was prepared by dissolving 100 mg of FDA in 20 ml of acetone to give a final concentration of 5 mg per ml. A stock solution of EB (Sigma, U.S.A.) was prepared by dissolving 20 mg of EB in 10 ml of HBSS, pH 7.4, containing 0.05% Tween-80 to give a final concentration of 2 mg per ml. One milliliter volumes of FDA and EB were distributed to 13 x 100 mm screw cap test tubes and stored at -20°C in a dark place. These solutions were stable for two years and stored for working solution. A fresh working solution of FDA was prepared daily by diluting the FDA stock solution 1: 10 in acetone to get 500 µg per ml. A 0.02 ml volume of the diluted FDA solution was added to 5.0 ml of HBSS with 0.05% Tween-80 to make 2 µg per ml. A 0.01 ml volume of EB stock solution was then added to the same 5.0 ml of HBSS, containing the FDA, giving a final EB concentration of 4 µg per ml.

A 0.5ml volume of the FDA-EB working solution and phytochemicals of crude extract were added to a 1.0 ml aliquot of single-cell suspension and incubated for 1 min at room temperature. After preparing the wet mount and sealing to prevent evaporation, the suspension was observed in 950x Leitz Dialux microscope. Cells were counted in replicate and differentiated on the basis of their color. Green cells were considered to be live and red-stained cells were considered to be dead. Percent viability was calculated by dividing the number of green cells by the total number of cells observed and multiplying by 100.

Viable counts were performed in Neubauer-Hausser chamber. The inoculum was evenly distributed on the agar surface with a flamed L-shaped glass rod. Inoculated plates were incubated at 37°C for three days before plate counts were made. 1 ml volume of the original single-cell suspension was autoclaved at 121°C for 10 min to kill the bacteria. 0.5 ml volume of the FDA-EB working solution and phytochemicals of crude extract were added to the killed single-cell suspension and both chambers of the hemacytometer filled with stained-cell suspension. Bacterial counts were performed under incident UV illumination at 400x magnification after 10-min incubation at room temperature.

2.2.6 Osmoprotection by exogenous glycine betaine: Glycine betaine can protect plants from salt and freezing stress[13,14]. To assess the feasibility of glycine betaine as an osmoprotectant in *Borreria articularis* Linn., exogenously supplied glycine betaine was tested as prevention of hydroponically growing *Borreria articularis* Linn. in salt stress. To measure the effect of salt on plant growth we compared the 3-week-old grown plant in the presence or absence of 0.2 M NaCI.

Plants grown in the absence of NaCI reached an average height of 5.52 cm, plants grown in the presence of NaCI only reached a height of 2.91 cm. This inhibitory effect of NaCI could be improved by addition of glycine betaine. Supply of glycine betaine, with a 20 mM final concentration, to the growth medium together with NaCI resulted in plants with an average height of 5.21. These preliminary results indicated that exogenous glycine betaine can protect uncontaminated *Borreria articularis Linn.* exposed to inhibitory concentrations of NaCI and recommend that introduction of the betaine biosynthetic pathway to *Borreria articularis Linn.* could result in enhanced stress tolerance.

2.2.7 BADH Enzyme target antimicrobial Screening: *P. aeruginosa* PAO1 was provided by ICDDR'B was used in this experiment. Cells were grown aerobically at 37°C in liquid media. The basal medium was used to grow the cells as the M63 minimal medium. 20 mM choline was used as carbon and nitrogen source for maximum induction of BADH. The medium (1.8 litre) was inoculated with a seed culture (36 ml) in the log phase and was grown on a gyratory shaker (150 rpm) at 37°C until the stationary phase was reached. The cells from 1.8 liters of culture medium were harvested by centrifugation at 3,000X 3 g for 10 min and then resuspended in 90 ml of 50 mM potassium phosphate buffer (pH 6.5) containing 0.1 mM EDTA and 20 mM b-mercaptoethanol (buffer A).

The purification process was conducted at 4°C. The disintegrated resuspended cells by sonic oscillation (90 s at 60 W) in a Branson Sonifier Cell Disruptor were centrifuged at 14500x 3 g for 30 min. 20% (w/v) sucrose (buffer B) was placed into the supernatant (cell extract) and then applied to a Q-Sepharose Fast Flow column (1.8 X 6.5 cm) equilibrated with buffer B. The column was washed with the buffer B and the enzyme was eluted with 110 ml of a linear salt gradient of 0 to 250 mM KCl in buffer B at a flow rate of 1 ml/min. Fractions with enzyme activity were collected and the pH was adjusted to 6.0 with diluted HCl. after pH adjustment, the enzyme was applied to a 29, 59-ADP–Sepharose column (1.1 X 3.5 cm) equilibrated with buffer C (10 mM potassium phosphate with pH 6.0 containing 5 mM DTT, 20% (w/v) Alkaloids, Glycoside, Reducing sugar, Gums and Saponin of plant extract, 20% (w/v) sucrose, 0.1 mM EDTA, and 25 mM KCl). After washing with buffer C (50 ml), the enzyme was eluted at a flow rate of 1 ml/min with 85 ml of a linear pH gradient, from 6.0 to 8.0 of buffer C. The enzyme eluted at pH 6. Fractions with enzyme activity were collected, aliquoted, and stored at 220°C.

During the purification procedure, the BADH activity was assayed spectrophotometrically by monitoring the absorbance at 340 nm (NADPH formation) in a mixture (0.5 ml) consisting of 1.0 mM betaine aldehyde and 0.3 mM NADP1 in a 100 mM potassium phosphate buffer, pH 8.0 (standard assay). Shimadzu spectrophotometer (Japan) equipped was used at 30°C in 1.0-cm-path-length cuvettes for the assays. All assays were initiated by addition of the enzyme. The initial rate of betaine aldehyde oxidation was proportional to the enzyme concentration over a range of 0.06 to 2.3 mg of protein per ml of reaction mixture. Each determination was performed twice. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 mmol of NADPH per min in our standard assay.

3. Results and Discussion

3.1 Phytochemical group Analysis: Phytochemical study showed the presence of alkaloids, glycosides, steroids, gums, saponin and reducing sugar were presented in the two extracts and found absence of other components (Table 1).

Table 1: Phytochemical composition of the *Borreria articularies* (Linn.)

Secondary metabolites	Extract	
	Methanol	Ethanol
Alkaloid	+	+
Glycoside	+	+
Steroids	+	±
Gums	±	±
Reducing sugar	+	+
Tannins	-	-
Flavonoids	-	-
Saponin	-	±

(+) = Presence, (-) = Absence, (±) = medium

3.2 Disc Diffusion assay: Extracts were tested for antibacterial activities on gram positive (*Staphylococcus aureus)* and gram-negative (*Escherichia coli)* bacteria using disc diffusion method. 1 mg of the extracts was applied in each disc. A standard antibiotic disc of Amoxicillin (30 µg/disc) was used for comparison of antibacterial activity. The antibacterial activities of the extracts were shown in table 2. Methanol extract of the plant showed low-level activity on the test organisms.

Table 2: In-Vitro antibacterial activities of *Borreria articularis* at 2 days

Organism	Zones of inhibition (mm)			Percent inhibition
	Methanol extract (1mg/disc)	Ethyl Alcohol extract (1mg/disc)	Amoxicillin (30µg/disc)	
Staphylococcus aureus	1.3	Nil	2.8	53.57%
Escherichia coli	1.5	Nil	2.8	46.43%

3.3 *In Vitro* Antifungal screening in yeast: The extracts were tested for the antifungal properties on human pathogenic yeast (*Candida albicans)*. Clotrimazole (30µg/disc) was used as Standard. Results obtained from the screening were shown in the following table 3. The extracts did not show antifungal activities.

Table 3: *In Vitro* Antifungal activities of *Borreria articularis* Linn. in the yeast

Name of the yeast	Methanol (1mg/disc)	Ethanol (1mg/disc)	Clotrimazole (30µg/disc)
Candida albicans	No activity	No activity	Strong activity

3.4 BADH activity assay: Growth of *P. aeruginosa* on choline showed an induction of significant intracellular levels of BADH activity (around 1 U per mg of protein in the cell extract), which was completely absent in cells grown in the standard glucose medium and in the plant phytochemicals. This may be due to induction of the enzyme by choline in *Pseudomonas*[15] and other bacteria[16,17]. Inhibition of BADH in the phyto constituents may be for the blocking choline degradation, abolishing synthesis of the osmoprotectant glycine betaine, or accumulating the BADH substrate, betaine aldehyde[18,19,20]. The presence of glycine betaine or choline greatly increases the ability of P. aeruginosa to thrive under osmotic stress[20, 21, 22]. Also, the virulence of P. aeruginosa is associated with its ability to adapt to osmotic stress[23] and with the expression of phospholipase C[24], produced from bacteria to produce choline from phosphatidylcholine. *P. aeruginosa* produces glycine betaine through BADH-catalyzed reaction in the presence of NADH and NADPH when the bacterium is growing in choline or choline precursors[25]. In this regard BADH might be a key enzyme to grow the pathogen. BADH activity is critical for the growth of *P. aeruginosa* in infection. BADH metabolic role is the synthesis of the osmoprotectant, such as E. coli[26], Bacillus subtilis[27], amaranth leaves[28], and porcine kidney[29].

The activity BADH was determined in the presence of NaCl, NH4Cl, glucose, choline, alkaloids, glycosides, reducing sugar, gum and saponin (Table 4). The activity was reduced in the phytochemicals. The significant amount of BADH activity in *P. aeruginosa* cells grown on glucose plus choline, the finding that BADH activity is tolerant to salt that is also varied and found in earlier study[25]. It suggests may play an important physiological role in the infection sites of human and plant cells thru glycine betaine synthesis *in vivo*.

Table 4: Effect of methanol-ethanol extract of plant, glucose, choline and salt on the expression of BADH activity in *P. aeruginosa*

Culture conditions	BADH activity (mU/mg protein)
11.1 mM glucose/15 mM NH4Cl	8.0
11.1 mM glucose/15 mM NH4Cl/0.4 M NaCl	11.0
Alkaloids/15 mM NH4Cl	15.0
Alkaloids /15 mM NH4Cl/0.4 M NaCl	22.0
Alkaloids /11.1 mM glucose	5.0
Alkaloids/20 mM choline	27.0
Alkaloids/20 mM choline/0.4 M NaCl	30.0
Glycoside/15 mM NH4Cl	12.0
Glycoside/15 mM NH4Cl/0.4 M NaCl	18.0
Glycoside /11.1 mM glucose	12.0
Glycoside/20 mM choline	10.0
Glycoside/20 mM choline/0.4 M NaCl	15.0
Reducing sugar/15 mM NH4Cl	16.0
Reducing sugar/15 mM NH4Cl/0.4 M NaCl	12.0
Reducing sugar /11.1 mM glucose	12.0
Reducing sugar/20 mM choline	13.0
Reducing sugar/20 mM choline/0.4 M NaCl	13.0
Gums/15 mM NH4Cl	8.0
Gums/15 mM NH4Cl/0.4 M NaCl	8.5
Gums /11.1 mM glucose	10.0
Gums/20 mM choline	11.0
Gums/20 mM choline/0.4 M NaCl	9.0
Saponin/15 mM NH4Cl	13.0
Saponin/15 mM NH4Cl/0.4 M NaCl	13.0
Saponin /11.1 mM glucose	10.0
Saponin/20 mM choline	9.0
Saponin/20 mM choline/0.4 M NaCl	14.0
20 mM choline	600
20 mM choline/0.4 M NaCl	302
20 mM choline/11.1 mM glucose	150
20 mM choline/11.1 mM glucose/0.4 M NaCl	170

3.5 Viability assay

A suspension of *M. smegmatis* was subdivided into 1.0 ml aliquots and exposed to FDA, EB, alkaloids, glycosides, reducing sugar, gum and saponin singly and in combination to determine effect in viability of the organisms during the staining procedure. After various periods of exposure, a 0.1 ml volume of cell suspension was removed from each tube, diluted in HBSS and plated on DAM, and plate counts were performed. FDA, EB had no effect upon the viability of the cells during an incubation period of up to 6 hr at room temperature (Table 5). Alkaloids, glycosides, reducing sugar, gum and saponin showed inhibition of most bacteria in the media (Table 5).

Table 5: Effect of phytochemicals of crude extract, fluorescein diacetate (FDA) and ethidium bromide (EB) on the viability of *Mycobacterium smegmatis* as determined by plate counts

Time (hr)	No of bacteria (x10) per ml of HBSS containing								
	Normal	FDA	EB	FDA-EB	Alkaloids	Glycosides	Reducing sugar	Gums	Saponin
0	2	2	3	2	1	0	0	1	0
3	3	3	3	2	0	0	0	1	1
6	2	3	3	2	0	0	0	1	1

Mycobacterial viability was compared to standard bacteriological plate counts using 18 hr to 27 hr DLM cultures at 37°C. A high degree of correlation ($p < 0.005$) was found between the two methods with an average difference of 20%. Higher percent viability may be due to a small amount of cell reclumping after single-cell suspensions preparation (Table 6). Two or more clumped cells may be grown in plating into a single colony and may give lower plate counts. The adherence of bacteria to the L-shaped spreading rod is the reason for the lower counts.

Table 6: comparison of *Mycobacterium smegmatis* cell viability in plating, FDA-EB and phytochemicals of crude extract

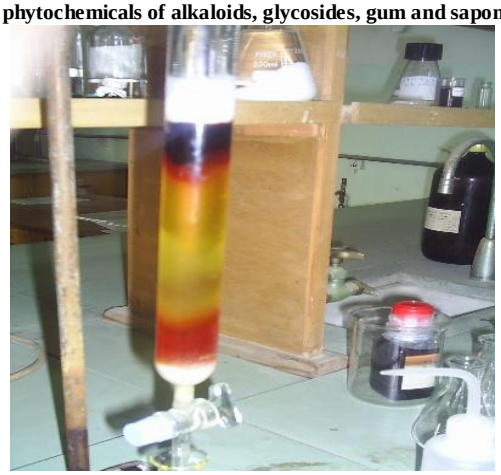
Organism	Culture age (hour)	Percent viability determined						
		Plating	FDA-EB	Alkaloids	Glycosides	Reducing sugar	Gums	Saponin
Mycobacterium smegmatis	22	95	95	85	89	90	90	85
	25	80	92	87	85	90	90	85
	27	80	92	85	88	89	87	89

After experiments, it was found that some cells were dual stained. These cells were characterized as thickness variable with green. It indicated acetyl esterase activity and diffusion to red-orange nuclear area as penetration of EB to confirm the cells with damaged cell membrane.

4. Conclusion

Taken together, *Borreria articularies Linn.* showed its antibacterial activities in the bacterial cell and enzyme. The TLC separated Phytochemicals of alkaloids, glycosides, reducing sugar, gum and saponin were isolated (Figure 2) to justify the antibacterial effects of these compounds. These compounds showed antibacterial activity in many published study, too so that according to results of this study, it can be targeted to discover a new antibiotic. On the other hand crude extracts did not show any anti-yeast properties due to absence of BADH enzyme. Further study is required to find the reason of this effect of *Borreria articularies Linn.*

Figure 2: Aliquoted phytochemicals of alkaloids, glycosides, gum and saponin in column chromatography



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