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Antimicrobial activity investigation of *Aegle marmelos, Couroupita guianesis, Manilkara hexandra,* Cow Urine and Dung.

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ABSTRACT

Most of medicinal plants have potential to inhibit the bacterial growth and that potential is known as antimicrobial activity (in case of bacteria, antibacterial). India is a rich source of plant variety. Medicinal plants are a source of great economic value all over the world. The most commonly plants are used in Ayurvedic treatment as well as oyher traditional systems of medicine. In this present study Aegle marmelos, Couroupita guianesis and Manilkara hexandra are used for investigate the antibacterial activity. Leaves extract made with 60% and 100% methanol were used for study. Fresh cow urine and processed cow dung (Dalang) were also investigated for their antibacterial activity. Leaves extract of all three plants with cow urine were also used for microbial activity. Well diffusion method was against some of pathogens but there were no activity showed with urine and dung.

Keywords: Antimicrobial activity, antibacterial, cow urine, cow dung

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INTRODUCTION

Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. There are about 45,000 medicinal plant species in India mainly spotted in Eastern Himalayas, Western Ghats and Andaman & Nicobar Island regions. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. It is estimated that about 80,000 species of plants are utilized in some form or other by the different systems of Indian medicine (http://medicinalpltsinindiamp.blogspot.in/). Medicinal plants are a source of great economic value all over the world. Natural plant products are an important source to control bacteria and pathogens. About 30% of drugs used worldwide are based on natural products. Plant products like herbs and spices as part of food, extract and powder have been used with varying success to cure and prevent diseases throughout history. Medicines made from plant parts are better because they are conventional, cheap, non-toxic, without side effects and readily available [1, 2]. In developing countries, the drugs are not only expensive and inadequate for the treatment of diseases, but also have side effects. Therefore there is a need to develop new strategy to control microbial infections [3]. Nowadays, about 70% of the bacteria that cause infections are resistant to at least one of the drugs which is most commonly used for treatment [4]. Bacteria may cause numerous diseases ranging from basic diseases found in daily life such as typhus fever (caused by Rickettsia bacteria), diarrhea, acne, skin papilloma etc. Traditional medicine has been improved and used in developing countries as an alternative cost effective therapy and an alternative to some commercial drugs [5].

In Indian Vedas, cow is considered the most valuable and religious animal of Hindus. In India, cows are very important animal and useful in agriculture and dairy industry. The cow urine has great pharmacological properties which are well documented. Cow urine exhibits both antioxidant and antimicrobial activities, which has been confirmed by a study of Edwin et al. [6]. Therefore, the distilled cow urine is being marketed by some of charitable religious foundations, Gaushalas etc. Extract of certain plants as well as cow urine in combination is found to possess marked inhibitory effect on human and plant pathogens [7-10].

The cow dung has been used as organic fertilizer and in the production of biogas. The evaporated extract of cow dung is called “Dalang” or “Dalam” in northeast Nigeria and in some part of Northem Cameroun and has been used as soup condiment and in treatment of infections [11]. The present study investigates antimicrobial properties of some of medicinal plants *Aegle marmelos* (commonly known as bael, Bengal quince, golden apple, stone apple, wood apple, bili), *Couroupita guianesis* (known by several common names, including cannonball tree), *Manilkara hexandra* (is native to much of south Asia China) , and cow urine and dung.

MATERIALS AND METHODS

Collection of Bacterial culture

Bacterial cultures of *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* on agar slant for our work, were provided by the Adarsh Science College, Patan and Biotechnology Department, Municipal Arts and Urban Science College, Mehsana (Gujarat) for the present studies.

Collection of plant, cow urine and dung

Leaves of plants species; *Aegle marmelos, Couroupita guianesis and Manilkara hexandra* were collected and dried under shed. Cow urine and dung was collected in early morning from preselected healthy Kankrej cow that is indigenous cattle breed of Gujarat. Cow dung was also put for shed dried.

Preparation of plant extract using methanol

Shed dried leaves were grinded to make fine powder. Out of which 20 gm powder of each plant was dissolved in 100 ml methanol (60% and 100% for two different extract). The mixtures were mixed vigorously and kept them at room temperature (27 – 32 ^{O}C). After 20 days mixtures were filtered by Whatman filter paper and put for air dried at room temperature. Dried material was weighed and reconstituted in 20%

Dimethyl Sulfoxide (DMSO) to get final concentration at the rate of 200mg / 200µl. These solutions were then stored in refrigerator in amber tubes until used.

Preparation of mixture of Cow Urine and plant extract: 10 gm dried powder of each plant was dissolved in 100ml fresh urine mixed for one hr on magnet stirrer and then were incubated 20 days at room temperature. Every day it was mixed twice by inverting the flask and vigorously shaking. After incubation, mixture was filtered with Whatman filter paper and filtrate was stored in amber bottle in refrigerator for further use.

Cow Dung extract: approximately 200 to 250 gm of dung was taken and dried under sunlight. After complete dry, it was burnt to make ash. Ash was dissolved in 500ml of water and mixed on stirrer for 30 minutes. After mixing, it was boiled for 2-3 hrs to evaporate 95% of water to obtain precipitate form. Later, it was air dried to make dry powder. Out of which, 1gm of powder was dissolved in 100ml distilled water, incubated for 24 hr, filtered after incubation and filtrate was stored for the study.

Preparation of penicillin: for positive control, 400mg tablet of Penicillin G potassium was dissolved in 10 ml sterile distilled water. That was further diluted to make a concentration of 1mg/ 200µl/ well.

Culture & Media preparation

All bacterial culture was maintained on nutrient agar. Inoculums were prepared by dissolving separated colonies in 1ml sterile distilled water. These suspensions were compared with 0.5 scale of Mc Farland's turbidity standard and maintained same. A small volume, 0.2 ml of each suspension was mixed with 10 ml of Muller Hinton agar (Himedia). Muller Hinton agar plates were prepared by pouring 20ml plain Muller Hinton agar and then adding 10 ml agar with 0.2 ml bacterial suspension for the study. Wells were made with sterile cork borer on cultural plates. Approximately 200µl (@1mg/1µl) of all antimicrobials components; leaves extracts, cow urine and dung extracts were loaded in the defined wells, plates were incubated at 37º C for 24 hr, zone of inhibition were measured and noted.

RESULT AND DISCUSSION

The extract from different plants, cow urine and cow dung were used to determine for their antimicrobial activity against three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis)* and two gram negative (*Pseudomonas aeruginosa, Escherichia coli)* bacteria. The whole experiment was repeated twice to avoid major errors and factors. The average of both results of antimicrobial activity was considered which are tabulated below.

Table: Indicating zone of inhibition by various antimicrobial extractions and penicillin in mm

	Plant Extract with 100% Methanol				Plant Extract with 60% Methanol				Plant Extract with Cow Urine				Cow Urine & Dung		
	B	N	R	PC	B	N	R	PC	B	N	R	PC	U	D	PC
Staphylococcus Aureus	2	8	4	20	0	6	3	21	0	0	0	21	0	0	25
Staphylococcus Epidermidis	2	7	6	23	0	9	2	22	0	3	2	22	0	0	24
Bacillus Subtillis	0	3	1	16	0	4	2	17	0	0	0	17	0	0	16
Pseudomonas Aeruginosa	0	7	2	0	6	7	3	0	0	0	0	0	0	0	0
Escherichia Coli	0	7	1	10	1	3	1	7	0	0	0	9	0	0	8

Where: B – Bili *(Aegle Marmelos),* **N** – Nagchampa *(Couropita Guianensis),* **R** – Rayan *(Manilkara Hexandra),* **U** – Cow Urine, **D** – Cow Dung, **PC** – Positive Control (Penicillin)

The bar graphs mentioned below indicate antimicrobial activity of penicillin (positive control), plant extraction using 100% methanol respectively (fig 1). The X-axis indicates name of bacteria whereas Y-axis indicates zones of inhibition in mm. The plant extracts of *Aegle Marmelos* with 100% methanol indicates that was poorly effective on two bacterial species; *S. Aureus* and *S. Epidermidis* whereas no effect on other

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bacteria. Plant extract of *Couropita Guianensis* was effective for all five bacterial species. Similarly, extract of *Manilkara Hexandra* was effective for antimicrobial actives against all five strains of bacteria but lesser than extraction of *Couropita Guianensis (*fig 1 & 5). Positive control or Penicillin was highly effective against all bacterial species.

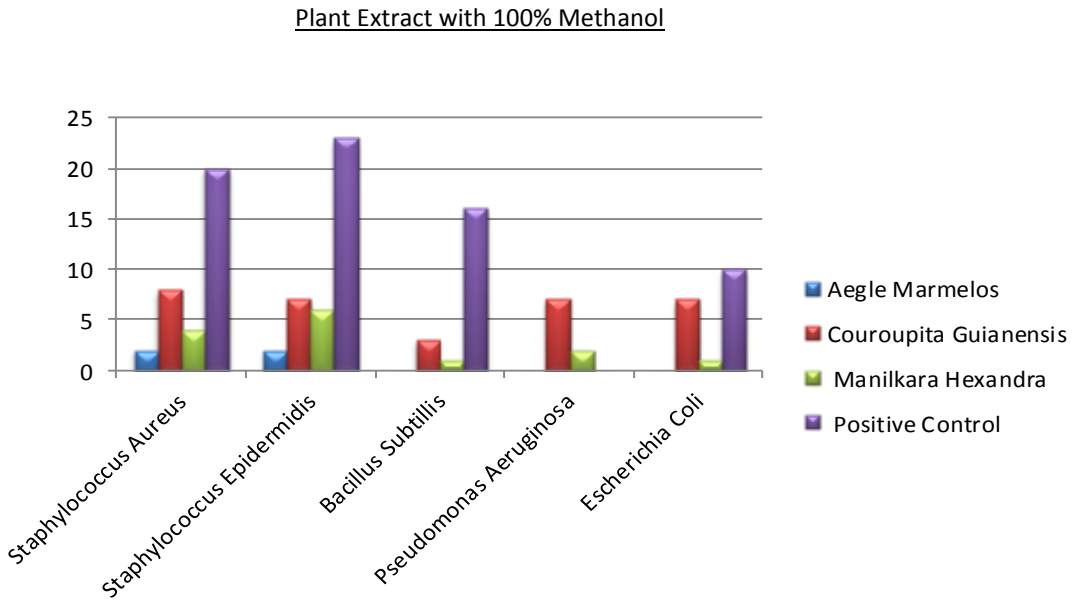

Figure 1: Bar graph indicates the effect of plant extraction with 100% methanol on various species of bacteria

The bar graphs mentioned below indicate antimicrobial activity of penicillin (positive control) and plant extract with 60% methanol. Similarly, the plant extracts of *Aegle Marmelos* with 60% methanol was poorly effective on two bacterial species; *S. Aeruginosa* and *E. Coli* whereas no effect on other bacteria. However, plant extract of *Couropita Guianensis* and *Manilkara Hexandra* was effective for antimicrobial actives against all five strains of bacteria (fig 2 & 6).

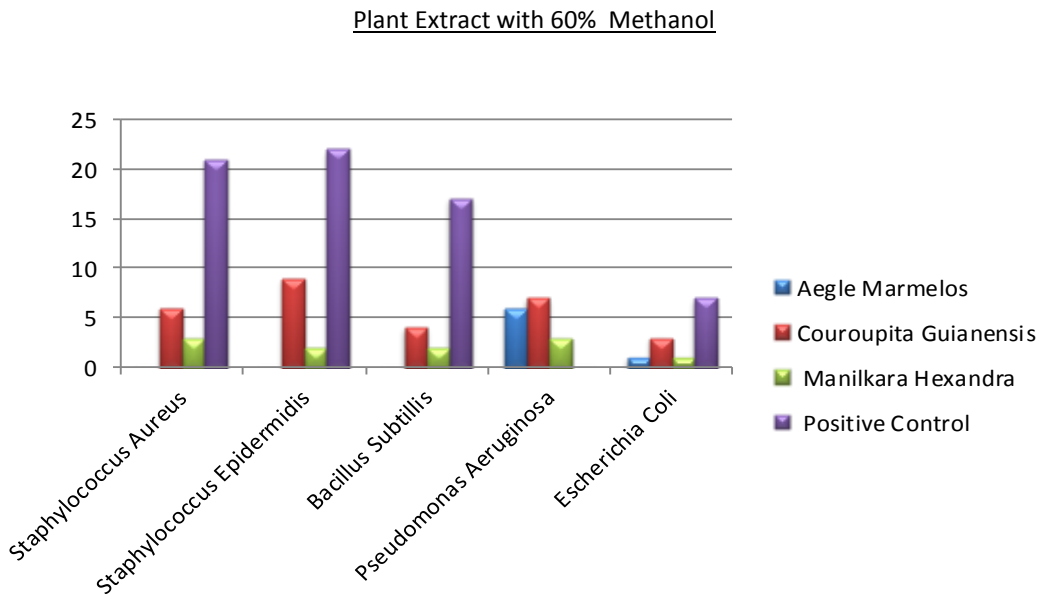

Figure 2: Bar graph indicates the effect of plant extraction with 60% methanol on various species of bacteria

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Plant extraction with the help of cow urine was not effective against all bacteria except *S. Epidermidis* as indicated in bar graph (fig- 3 & 7). Similarly, cow urine and dung was not at all effective against any bacteria (fig- 4 & 7)

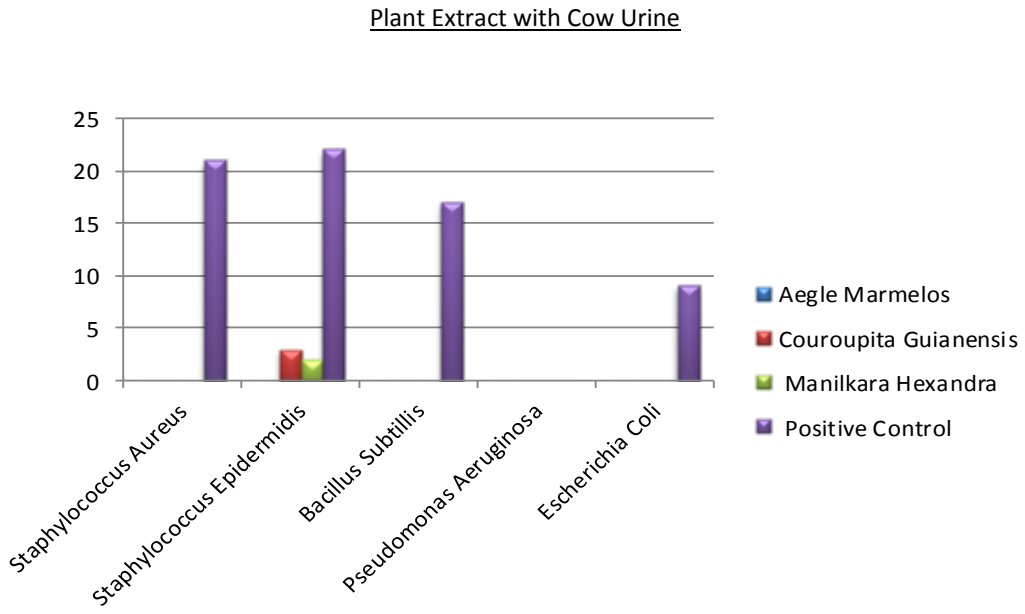

Figure 3: Bar graph indicates the effect of plant extraction with cow urine on various species of bacteria

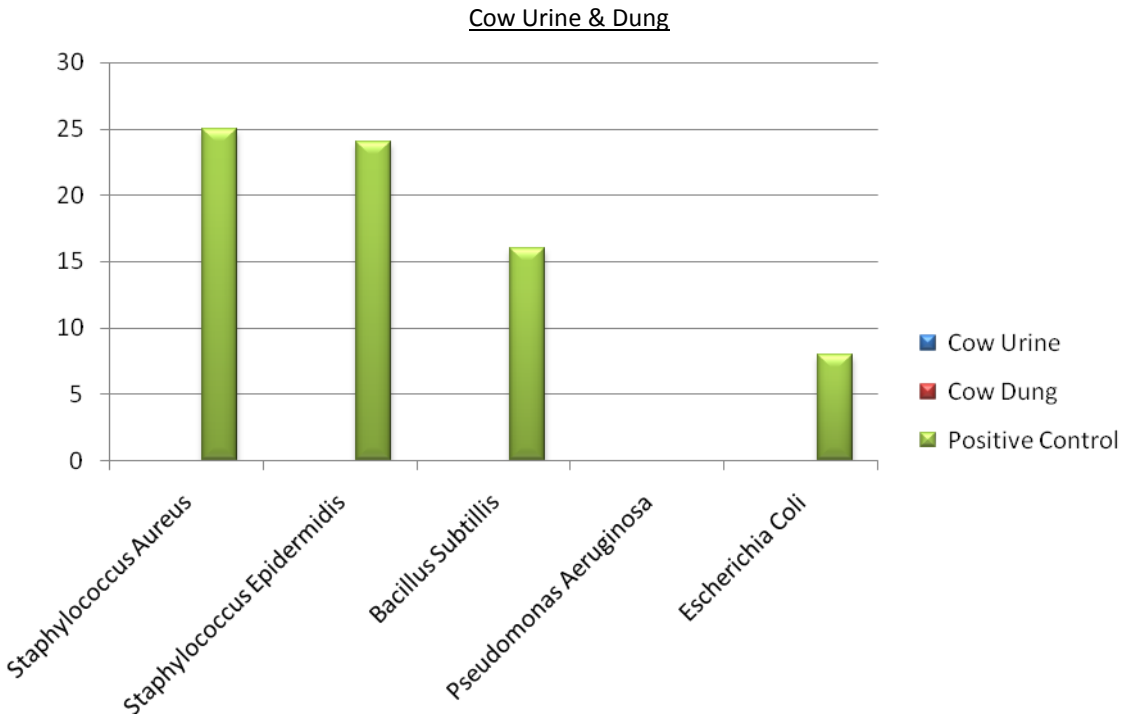

Figure 4: Bar graph indicates the effect of cow urine and dung on various species of bacteria

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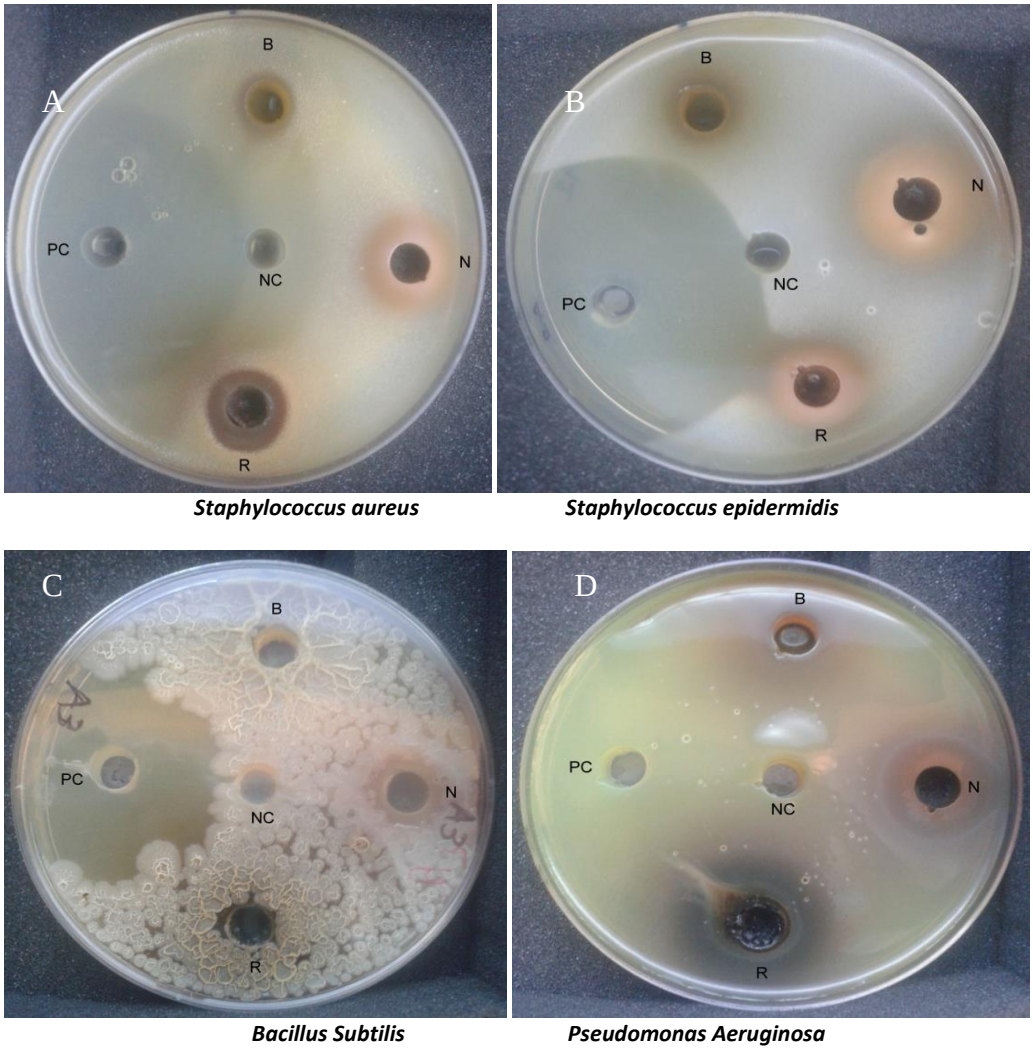
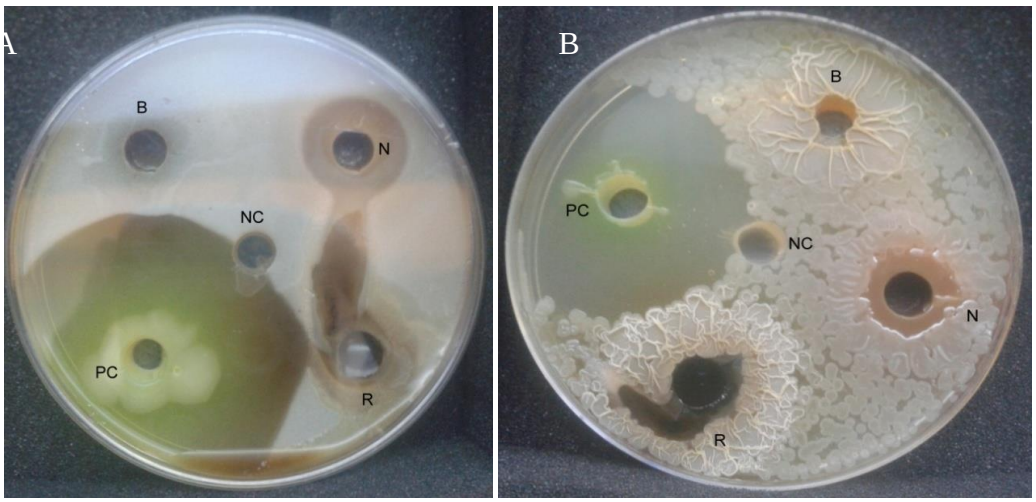


Staphylococcus aureus *Staphylococcus epidermidis*

Bacillus Subtilis *Pseudomonas Aeruginosa*

Figure 5: Plant Extract with 100% Methanol. Zone of inhibition due to plant extraction of Bili (B), Nagchampa (N), Rayan (R). Whereas, PC and NC indicate Positive Control (Penicillin) and Negative Control respectively.



Staphylococcus epidermidis *Bacillus subtilis*

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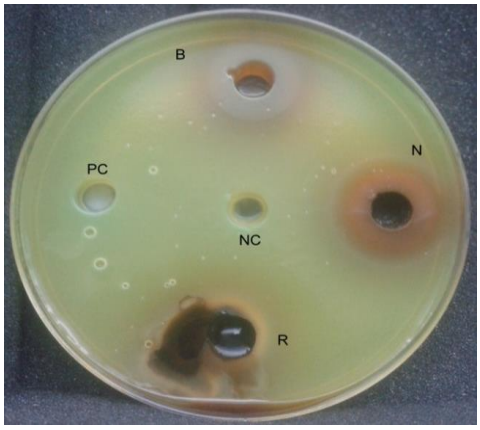
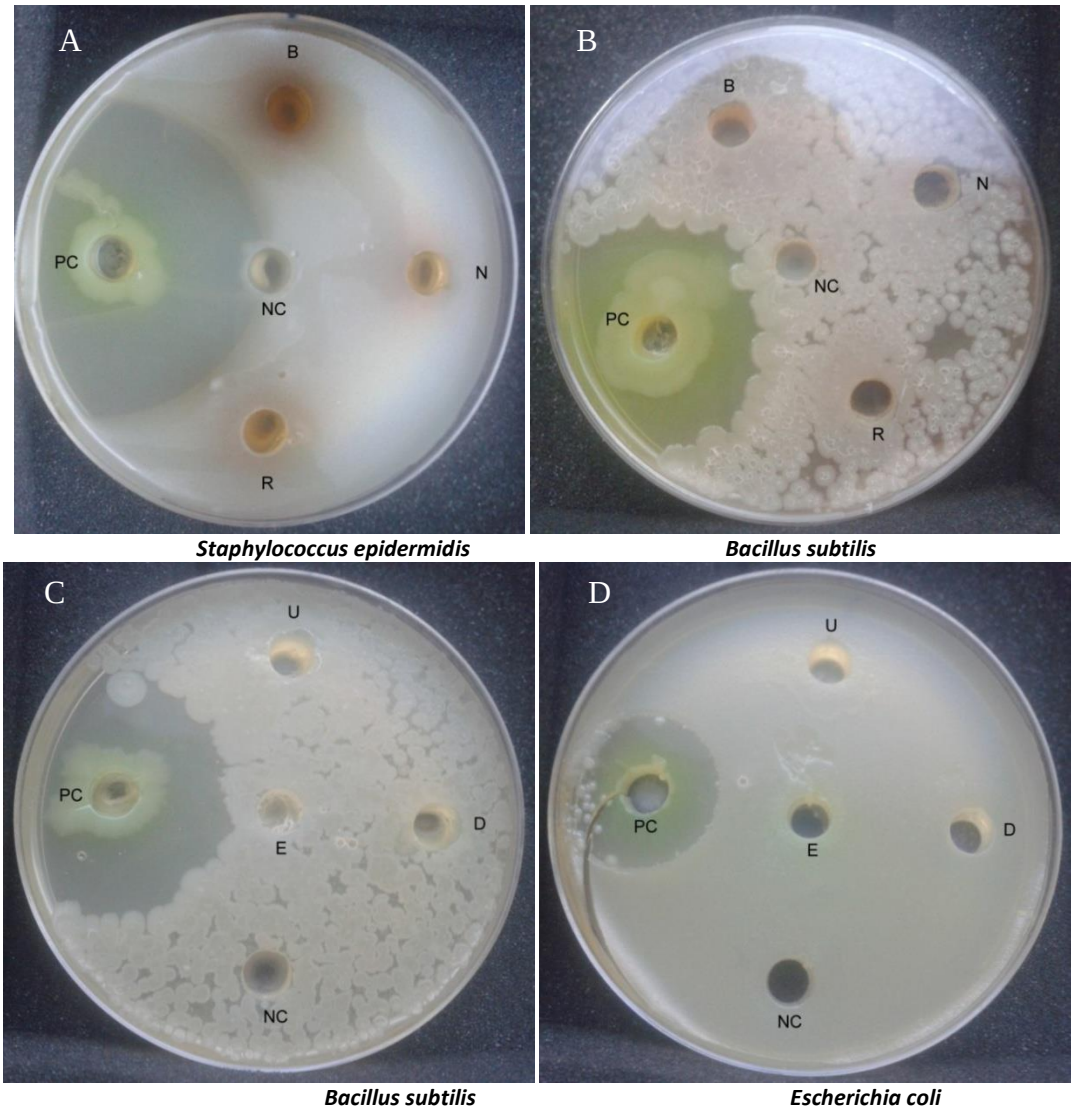


Pseudomonas aeruginosa

Figure 6: Plant Extract with 60% Methanol. Zone of inhibition indicated due to plant extraction of Bili (B), Nagchampa (N), Rayan (R). Whereas, PC and NC indicate Positive Control (Penicillin) and Negative Control respectively. Positive plates are shown in figure. However, all five bacteria under studies were tested.



Staphylococcus epidermidis *Bacillus subtilis*

Bacillus subtilis *Escherichia coli*

Figure 7: Plant leaves powder extract with cow urine (A & B) and extraction with cow urine and cow dung (C & D). Whereas B (Bili), N (Nagchampa), R (Rayan), PC (Positive Control (Penicillin), NC (Negative Control). Positive plates are shown in figure. However, all five bacteria under studies were tested.

DISCUSSION

In present study we have found antibacterial activity of plant extract with methanol solvent against gram positive & gram negative bacteria but not encouraging as compared to penicillin. An earlier study exhibited good results for same plant species against same organisms [12]. They considered three different solvent extracts like petroleum ether, chloroform and methanol for their activity. The results of their studies indicated that petroleum ether having highest activity than chloroform, and chloroform has more activity than methanol extract. Methanol in that case was poor solvent therefore poor or no activities of plant extracts against bacterial species could be the main reason. Other study of Aegle marmelos for its leaf, fruit and peel was carried out by Pandey and Mishra [13] wherein, they observed that fruits having maximum antibacterial activity compare to peels and leaves. Their work shows that ethanol and ethyl acetate extract are more superior to methanol and hot water. Das et al, [14] also checked ripen fruits for activity against bacteria and observed good inhibition zone. Our investigations in present studies show the good antibacterial activity of *Couroupita guianesis* when extract with 60% & 100% methanol for most of the pathogen used in study. Many studies show that *C. Guianesis* have antibacterial and wound healing potential. Umachigi et al, [15] reported antibacterial and wound healing potential of this plant with 50% ethanol extract. Gousia et al, [16] reported that *C. Guianesis* possess antibiotic, antiseptic and analgesic qualities. Kavitha et al., [17] noted the presence of phytochemicals in *C. Guianesis* leaf extract with methanol. The presence of carbohydrates, tennins, phenols, saponins, gums & mycilage and flavonoids were noted in leaf extract. Some researchers also show the use of different parts of the *C. Guianesis* for antimicrobial activity. Fruit chloroform extract was tested for antimicrobial and antimycobacterial activity by Al-Dhabi et al., [18] and they stated that fruit extract having good antimicrobial activity but very low antimycobacterial activity. Flower extracts in methanol having antimicrobial activity, which is effective against clinical pathogens such as *P. aeruginosa, S. aureus, Vibrio mimicus* & *Vibrio harveyi* reported by Ramalakshmi et al., [19].

A third plant species *Manilkara hexandra* also having a antibacterial activity to little extent. Due to paucity of literature on the extraction of this plant, it was not possible to compare with other research work. However, Gopalkrishnan et al., [20] reported that *M. hexandra* stem bark extract with water, alcohol and chloroform having most phytochemicals properties.

Many researchers have published that cow urine and cow dung having antioxidant and antimicrobial activity [21-23], but our result showed no activity for urine and dung against any test organisms. It is recommended such plants extraction with different solvents may also be studied for further comparison.

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