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EVALUATION OF ANTIBACTERIAL ACTIVITY OF INDIGENOUS MEDICINAL PLANTS IN BANGLADESH AGAINST SOME SELECTED PATHOGENIC STRAINS

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Abstract

In developing countries, a wide variety of natural products are used as herbal medicine in the treatment of many common infections. Due to the association of pharmacological activities and presence of several constituents in medicinal plants, these are used as worldwide. This study gives a bird's eye view mainly on the antibacterial activity of methanolic leaf extracts of Deshi Neem (*Azadirachta indica*), Wild-type Neem (*A. indica*), German lota (*M. michranta*) and Telakochu (*Coccinia grandis*) against both gram-positive and gram-negative bacteria. Zone of Inhibition (ZOI) is used to determine the effect by agar well diffusion method. Four different concentrations 50, 100, 200 and 400 (µg/ml) of the extract is applied against some selected pathogens causing human diseases. Standard antibiotics were used to compare the antibacterial activity with the experimental leaf extracts. Methanolic extract of leaf demonstrated maximum inhibition zone on *S. aureus*, and *V. Parahaemolyticus* followed by other bacterial species. In this study, it is also predicted that these leaf extracts showed no effect on *E. coli* and *P. aeruginosa* and the highest zone of inhibition is about 26±0.33 mm and 27±0.33 mm against *V. parahaemolyticus*. The lowest one is against *K. pneumoniae*, and the zone of inhibition is about 8.67±0.33 mm, whereas the zone of in the inhibition was observed in the case of standard antibiotics from 9.0±0.32 to 22.3±0.36 mm. This study showed that the extract of tested plants possesses antibacterial activity against pathogenic strains. The extracts showed maximum inhibition zone on *S. aureus* and *V. parahaemolyticus* followed by other bacterial species. The inhibition effects of Neem are higher than those of antibiotics. The experimented plants extracts would be possible to use a traditional medicine instead of antibiotics.

Keywords: *antibacterial activity, methanolic extract, pathogenic strain, traditional medicinal plants*

Introduction

At present for the treatment of a wide range of infectious diseases, many potent antibiotics/chemotherapeutics are available. Further, many of the antibiotics are cost-effective and significant side effects, some of which are dangerous. For this, there is an urgent need for search and development of novel therapeutically active antibiotic drugs from natural/herbal sources [1]. Most of the drugs today are obtained from natural sources orsemi-synthetic derivatives, natural products used in the traditional systems of medicine. Thus, it is a logical approach to drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to the pharmaceutical or biological test. A sustainable number of new antibiotics introduced in the market are attained from natural or semi-synthetic sources [2]. The plant extracts of *P. granatum* and *S. aromaticum*have been proved to control food poisoning disease caused by *B. cereus, S. aureus, E. coli, S. type* and *P. aeruginosa* [3]. Antimicrobial compounds derived from plants were used for centuries in food preservation. Egyptians, Chinese, and Indians used spices and essential oils since ancient time. Some of the spices such as mint, garlic, and ginger are still practiced in alternative health remedies in India [4, 5]. Traditional herbal medicine has been a constant source of substances for the treatment of a variety of diseases [6]. About 85% of the traditional herbal medicines used for primary health care is derived from plants. Around 70% of the population in Canada use herbal medicine traditionally [7]. In addition 47% of the population in England use traditional herbal medicine derived from plants [8]. Moreover, in Latin America, the WHO regional office for the Americas reports that 71% of the population in Chile and 40% of the population in Colombia use traditional herbal medicine [9]. In India, the native people utilize a variety of herbals from the plants for the effective treatment of various maladies [10]. In Africa, a traditional herbal medicine derived from plants forms an integral part of life in many indigenous communities as a readily available alternative to allopathic medicines [11]. Plants have been an indispensable source of both preventive and curative traditional herbal medicinal preparations for many people in Africa. In Kenya, access to affordable health care is still generally unavailable for the average Kenyan, and 90% of the population has used the traditional herbal medicine at least once for various conditions [12].

The neem tree *A. indica* A. Juss. (Meliaceae) is a tropical evergreen related to mahogany. Native to East India and Burma, it grows in much of Southeast Asia and West Africa. *A. indica*belongs to the family Meliaceae, commonly known as neem. It is used in traditional medicine as a source of many therapeutic agents. Leaf, bark, and the seed of neem are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles, andCoxsackie B viruses [13]. More or less every part of the tree is bitter and finds application in indigenous medicine. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activity [14]. The extract from bark, leaves, fruits, and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children [15].

A decoction of the stem and leaves is used as a remedy for children's clysters and to treat malaria and eczema. The leaves are an antidote, cholagogue, diuretic, and febrifuge. They are boiled, and the water drunk as an anti-menorrhagia. Juice from the macerated leaves is applied to persistent sores and bush-yaws. The macerated leaves are vigorously rubbed on the skin as a treatment for rashes [16]. The leaves are thought to have antiseptic and antimicrobial properties [17, 18].

C. grandis, Telakucha or ivy gourd, also called scarlet gourd and Kowai is a tropical vine. It is additionally exceptionally mainstream in the Indian Territory of West Bengal, known Kunduri in Bengali with prevalent Bengali cooking like Kunduri Posto in Southeast Asia. It is developed for its palatable youthful shoots and consumable natural products. *C. grandis* L., of the family Cucurbitaceae, is dispersed in tropical Asia, Africa and is regularly found in Bangladesh, Pakistan, India, and Srilanka [19].

C. grandis is a climber and trailer. The plant is utilized as a part of decoction for gonorrhea, diabetes and furthermore valuable in dropsical condition. It has antimicrobial, hypolipidemic, antimutagenic and hypoglycemic exercises [20-24]

Medicinal plants play a significant role in providing primary health care services to the people. They serve as important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines [25]. In purpose of protecting biodiversity, we have to think about the sustainable use of plant resources.

Methods

Collection of plant materials

Leaves of four plants were collected from the different region of Bangladesh. *C. grandis* (accession no. 46670)were collected from a village of Jessore district, *A. indica* L, (accession no. 44984) were collected from Khulna division, *M. Micrantha* (accession no. 46669) were collected from the campus of Jessore University of Science and Technology, and*A. indica*wild-type (accession no. 44984) same as local type due to due to the same genus, were collected from Shatkhira district. Samples were collected by maintaining the rules of our country although we need not any written formal way to manage the samples in our country.

Preparation of plant extracts

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant [26]. The leaf of the experimented plants was cut into small pieces and then clean and dried in the shade for two weeks. The plant parts were ground into a fine powder with the help of a suitable grinder and were stored in an airtight container and kept in a cool, dark and dry place. The powdered materials were dissolved in 80% methanol (1:10); 1 gm sample should be dissolved in 10 ml of solvent [27]. Mixtures were kept in sterilized beakers wrapped with aluminum foil and were then kept in 3 days at room temperature. After three days, mixtures were filtered through Whatman no. 1 filter paper and then evaporated by rotary evaporator.

Collection of bacterial strains

The antibacterial activity of each plant extract was evaluated using eight bacterial strains. Three strains of Gram-positive bacteria as *Staphylococcus aureus, Bacillus subtilis* and *Sarcina lutea* and other five strains of Gram-negative bacteria were *Eshiacheria coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Vibrio cholera,*and *Vibrio* parahaemolyticus. The bacterial strains were collected from the microbiology laboratory of Jessore University of Science and Technology. The inoculums were prepared by using nutrient broth media and for the growth of bacteria Mueller Hinton (MH) agar was used.

Standard antibiotics

TC ^{30}: Tetracycline (30 µg/disc); NA^{30}: Nalidixic acid (30 µg/disc); Amp ^{25}: Ampicillin (25 µg/disc); E ^{15}: Erythromycin (15 µg/disc) were used to compare the antibacterial activity with the experimental leaf extracts.

Antibacterial Test Analysis

With the help of a cotton swab stick, the bacterial strain was taken from the nutrient broth and gently swabbed whole Petri dishes over the Mueller Hinton agar media. The plates were kept sometimes for dry out the whole inoculums, and then with the help of Bohrer, four holes were created in each Petri dish. With the help of micropipette, each hole was filled with different doses of plant extract solution and then incubated at 37° C for 24 hours.

Determination of Zone of Inhibition (ZOI)

After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) against the test organisms around each of the extracts and the antibioticswith a measuring scale.

Statistical Analysis

Diameter zones of the inhibition of extracts are reported as a mean ± standard error.

Results

In the present study, the methanolic leaf extracts of *A. indica* (Deshi), *A. indica* (Wild-type), *M. michranta* and *C. grandis* showed inhibition zone on *B. subtilus*, *S. aureus, V. cholera, K. pneumonia*, and *S. lutea* and *V. Parahaemolyticus*. The inhibition zone of methanolic leaf extracts on these bacteria has shown in Table 1.

Methanolic leaf extracts exhibited maximum inhibition zone on *S. aureus* and *V. Parahaemolyticus* followed by other bacterial species. An antibiotic

sensitivity test is done to help to choose the antibiotic that will be most effective against microbial strains. The antimicrobial activity of four antibiotics against seven bacterial strains is given Table 2.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The vast research was done, and many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, anti molluscan and anti-inflammatory properties of plants. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects. The leaves of these examined medicinal plants possessed good antibacterial activity confirming the enormous potential of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. The extract of *A. indica* (Deshi), *A. indica* (Wild-type), *M. michranta* and *C. grandis* when used as a medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium. The alkaloids, glycosides, flavanoids, and saponins are antibiotic principles of plants. These antibiotic principles are the defensive mechanisms of the plants against pathogens. The methanol leaf extract of Neem (Deshi) showed inhibition zone on four out of eight bacterial strains such as *B. subtilus*, *S. aureus*, *V. cholera*, *K. pneumonia*, and *S. lutea* showed in Figure 1

Also, methanolic leaf extract of Neem exhibited maximum inhibition zone on *S. aureus* followed by other bacterial species. In this study, methanolic extract *of M. micrantha* demonstrated great potential effects against *B. subtillis, S. lutea, S. aureus, and K. pneumoniae* and *V. parahaemolyticus* among the all experimental bacterial strains showed in Figure 2.

All five pathogenic bacteria such as *E. coli, K. pneumoniae, S. lutea, B. subtillis*, and *P. aeruginosa* were tested to evaluate the antimicrobial potential of *C. grandis* leaf extracts. The methanolic extract with the concentration shows moderate antimicrobial activity against *S. lutea* and *K. pneumonia*. The inhibition zone of *S. lutea* with the concentration of 50, 100, 200 and 400 (mg/l) were 12.3±0.33, 13.4±0.33, 13.7±0.33 and 14.3±0.33 respectively.

On the other hand, the inhibition zone of *K. pneumoniae* with the concentration of 50, 100 and 200 were 10.0±0.22, 11.0±0.58, 8.67±0.33 and 0.0±0.0 respectively showed in Figure 3.

The concentration of 400 didn't show any inhibition zone in the field of *K. pneumoniae*. Among five bacterial strains, *A. indica* (Wild-type) show inhibition zone on B. subtillis and K. pneumonia. In this study *A. indica* (Deshi), *A. indica* (Wild-type), *M. michranta* and *C. grandis* leaf extract showed inhibitory effect on both gram-positive and gram-negative bacteria showed in Figure 4 and the zone of sensitivity test have been shown in the Figure 5.

It also found that methanolic leaf extracts of *A. indica* showed no effect on *E. coli* and *P. Aeruginosa* in this research work. But according to Panchal et al., methanolic extract of neem showed an average inhibitory zone diameter (2.0 cm, 2.2 cm, 2.4 cm, and 2.1 cm) on E. coli [28]. It is often reported that Gram-negative bacteria are more resistance to the plant-based organic extracts [29] because the hydrophilic cell wall structure of the Gram-negative bacteria is constituted essentially of a lipopolysaccharide (LPS) that blocks the penetration of hydrophobic oil and avoids the accumulation of organic extracts in target cell membrane [28].

Discussion

The plant extracts of *P. granatum* and *S. aromaticum* have been shown potential effect for controlling food poisoning and got the highest zone ofinhibition is 16.1±0.46 against *P. aeruginosa* by the extract of the plant *Punica granatum* [3]. Here, we also examined four antibiotics against the selected bacterial strains where TC30 showed higher antibiotic sensitivity than other antibiotics.The highest zone was observed about 22 mm and the lowest one was about 9 mm in the case of the standard.

Since ancient time, the medicinal plants and its ingredients have therapeutics implication and have been traditionally used worldwide especially in the Indian Subcontinent. This study showed that methanolic leaf extract from *A. indica* (Deshi), *A. India* (Wild-type), *M. michranta* and *C. grandis* possesses antibacterial activity against bacterial species which are pathogenic to human. The extracts showed inhibition zone against *B. subtilus,*

S. aureus, V. cholera, K. pneumonia, and *S. lutea*, and *V. parahaemolyticus* whereas no effects were found in *E. coli* and *P. aeruginosa*. Thus, the extract showed maximum inhibition zone on *S. aureus* and *V. parahaemolyticus* followed by other bacterial species. Following the result of antibiotics against these bacterial strains showed that the inhibition zone of Neem is higher than the zone of inhibition of these antibiotics. The extracts of the selected plants have been proved to be potentially effective against the selected gram-positive and gram-negative pathogenic bacterial strains and shown the killing ability of the mentioned strains is almost closer to the marketed antibiotics.

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References

1. Silva AR, Dissanayake DM, Chaturanga RK., Ranaweera B, Pathirana R, Ratnasooriya WD. Evaluation of in vitro antibacterial activity of some Sri Lankan medicinal plants. Int J Phamceut Res Allied Sci. 2015; 4(2): 54-57.

2. Hemalatha M, Thirumalai T, Saranya T, Elumalai EK., David E. A review on the antimicrobial efficacy of some traditional medicinal plants in Tamilnadu. J Acute Dis. 2013; 2(2): 99-105.

3. Mostafa AA, Al-Askar AA, Almaary K.S, Dawou, TM, Sholkamy EN, Bakri MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning disease. Saudi J of Biol Sci. 2018; 25: 361-366.

4. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010; 21(9): 1199–1218.

5. Dhiman R, Aggarwal NK, Kaur M. Comparative evaluation of antimicrobial activities of commonly used Indian spices against microbes associated with juices. Res J Micro. 2015; 10(4): 170–180.

6. Kunwar RM, Acharya RP, Bussmann RW, Medicinal plants in Nepal West Himalaya: Status, trade, use, and community management. J Ethnobotany Res Appl. 2010; 3(8): 89-106.

7. Health Canada, Drug and Health products. Nat Health Prod Regul. 2003; 13:(137). Food and drugs act. Available at www.hc-sc.gc.ca.

8. Thomas K.J, Nicholl JP, Coleman P. Use and expenditure on complementary medicine in England: a population-based study. J Complement Med. 2001; 9(1): 2-11.

9. World Health Organization. Beijing Declaration of Congress on traditional medicine in 2008, Beijing, China, Geneva; WHO.

10. Verma AK, Kumar M, Bussman RW. Medicinal plants in an urban environment: the medicinal flora of Benares. J Ethnobotany Ethnomed. 2007; 3(2): 35-47.

11. Wagate CG, Maria JM, Gakuyu DW, Nanyingi MO, Kareru J. Screening some Kenyan medicinal plants for antibacterial activity. J Med Res. 2010; 24: 150-153.

12. Chirchir. Medicinal and Therapeutic Potential of Medicinal Plants. Int J Sci Res Pub. 2006; 2(1): 113-118

13. Biswas K, Ishita C, Ranajit KB, Uday B. Biological activities and medicinal properties of Neem (*Azadirachta indica*). Curr Sci. 2002; 82: 1336-1345.

14. Kirtikar K.R, Basu BD. Indian Medicinal Plants International Book Distributors. Dehradun. 1987; 1: 536-541

15. Chattopadhyay RR, Chattopadhyay RN, Maitra SK. A possible mechanism of anti-inflammatory activity of *Azadirachta indica* leaf extract. Indian J Pharm, 1993; 25: 99-100.

16. DeFilipps RA, Maina SL, Crepin J. Medicinal Plants of the Guianas. Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 2004; 20013-7012.

17. Cambie RC, Ash J. Fijian Medicinal Plants. CSIRO, Melbourne, Australia. 1994; 1-275.

18. Weiner MA. Secrets of Fijian Medicine. 1984; 1-141.

19. Sastri BN. The Wealth of India, A Dictionary of Raw Material and Industrial Products, Publication and Information Directorate CSIR New Delhi. 1950; (2 & 8): 257 and 285-293.

20. Kumar P, Sudheeshi GS, Vijayalakshmi NR. Hypoglycemic effect of Cocciniaindica, mechanism of action. Planta Medica. 1997; 59(4): 330-332.

21. Kusamran WR, Tepsuwan A, Kupradinum P. Antimutagenic and anti-carcinogenic Potential of some Thai vegetables. Mutat Res. 1998; 402(1, 2): 247-258.

22. Chopra RN, Bose JP. *Cephalandra indica* (Telakucha) in diabetes. Indian J Med Res. 1925;13: 11-16.

23. Gupta SS. Pituitary diabetes. III. Effect of indigenous antidiabetic drugs against the acute hyperglycemic response of anterior pituitary extract in glucose-fed albino rats. Indian J Med Res. 1963; 51(4): 716-724.

24. Brahmachari HD, Augusti KT, Birla and Pilani. Orally effective hypoglycemic principles from Cocciniaindica. J Pharm Pharmacol. 1963; 15(6): 411-412.

25. Hasan M, Hossain ASK., Ali A, Alamgir MAN. Medicinal plant diversity in Chittagong, Bangladesh: A database of 100 medicinal plants. J Sci Innov Res. 2014; 3(5): 500-514.

26. Pandey VPS, Saroj K, Chapagain SK, Kazama F. A framework for measuring groundwater sustainability. Environment Sci Policy. 2011; 14: 396-407.

27. Panchal P, Bajaj H, Maheshwari S. Azadirachtaindica (NEEM): antibacterial effects against *Escherichia coli* and Salmonella. Guru Drone J Pharm Res. 2013; 1(1): 18-21.

28. Reynolds JEF. Martindale the extra pharmacopeia, 31st ed. London. Royal Pharmaceutical Society of Great Britain, 1996, Doi: http://doi.org/10.1136/bmj.313.7066 .1214a.

29. Bezic N, Skocibusic M, Dunkic V, Radonic A. Composition and antimicrobial activity of *Achillea clavennae* L. Essential Oil. Phytother. Res. 2003; 17(9): 1037-1040.

Table 1. In vitro activity of *A. indica* (Deshi), *A. india* (Wild type), *M. michranta* and *C. grandis* leaves in methanolic extract against opportunistic pathogens.

Plant		Dia meter of the zone of Inhibition (mm)							
	Conc.(mg/ml)	EO1	EO2	EO3	EO4	EO5	EO6	EO7	EO8
M. micrantha	50	13.3±0.33	0.0±0.0	13.0±0.11	0.0±0.0	13.7±0.33	0.0±0.0	20.3±0.33	0.0±0.0
	100	14.7± 0.33	0.0±0.0	16.0±0.33	0.0±0.0	18.0±0.33	17.7±0.33	23.7±0.33	0.0±0.0
	200	17.3±0.33	0.0±0.0	17.66±0.33	0.0±0.0	18.3±0.33	18.3±0.33	26.3±0.33	0.0±0.0
	400	20.6±0.33	0.0±0.0	20.66±0.33	0.0±0.0	20.7±0.33	24.3±0.33	27.7±0.33	0.0±0.0
A. indica (local)	50	13.3 ±0.33	0.0±0.0	12.0±0.57	0.0±0.0	14.7±0.33	23.7±0.33	0.0±0.0	0.0±0.0
	100	13.0±0.33	0.0±0.0	14.0±0.45	0.0±0.0	15.7±0.33	22.3±0.33	0.0±0.0	0.0±0.0
	200	16.0±0.32	0.0±0.0	15.33±0.33	0.0±0.0	16.7±0.33	0.0±0.0	0.0±0.0	11.3±0.33
	400	15.0±0.57	0.0±0.0	14.33±0.33	0.0±0.0	16.7±0.33	0.0±0.0	0.0±0.0	0.0±0.0
C. grandis	50	0.0±0.0	0.0±0.0	10.0±0.22	0.0±0.0	12.3±0.33	0.0±0.0	0.0±0.0	0.0±0.0
	100	0.0±0.0	0.0±0.0	11.0±0.58	0.0±0.0	13.4±0.33	0.0±0.0	0.0±0.0	0.0±0.0
	200	0.0±0.0	0.0±0.0	8.67±0.33	0.0±0.0	13.7±0.33	0.0±0.0	0.0±0.0	0.0±0.0
	400	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	14.3±0.33	0.0±0.0	0.0±0.0	0.0±0.0
A. indica (wild type)	50	11.3±0.33	0.0±0.0	13.0±0.58	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	100	12.33±0.33	0.0±0.0	15.0±0.58	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	200	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	400	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Data are means of three replicates (n=3) ± standard error; **EO= Experimental Organism; EO_1 = *B. Subtilis*, EO_2 = E. coli, EO_3 = *K. pneumonia*, EO_4 = *P. aeruginosa* , EO_5 = *S. lutea*, EO_6 = *S. aureus*, EO_7 = *V. parahaemolyticus*, EO_8 = *V. choalera*

Table 2. Antibacterial activity of some commercial antibiotics.

Standard (Antibiotics)	Zone of Inhibition (mm)			
	TC^{30}	NA^{30}	Amp^{25}	E^{15}
EO_1	20.7±0.21	9.3± 0.33	0.0±0.0	14.3± 0.22
EO_2	19.6±0.33	18.6± 0.23	0.0±0.0	16.3± 0.34
EO_3	20.0±0.23	9.7± 0.53	0.0±0.0	16.3± 0.24
EO_4	19.3±0.34	9.0± 0.32	0.0±0.0	14.3± 0.30
EO_5	21.3±0.23	20.6± 0.36	0.0±0.0	20.6± 0.56
EO_6	22.3±0.36	9.3± 0.11	0.0±0.0	16.3± 0.25
EO_7	19.3±0.24	18.6± 0.22	0.0±0.0	15.3± 0.33
EO_8	20.6±0.35	19.7± 0.25	0.0±0.0	16.3± 0.43

Data are means of three replicates (n=3) ± standard error; **EO_1 = *B. Subtilis*, EO_2 = *E. coli*, EO_3 = *K. pneumonia*, EO_4 = *P. aeruginosa* , EO_5 = *S. lutea*, EO_6 = *S. aureus*, EO_7 = *V. parahaemolyticus*,

Figure 1. Antibacterial activity of methanolic extract of *A. indica* (Deshi Neem) against A) *B. subtilis* , B) *E coli*, C) *K. pneumoniae*, D) *P. aeruginosa*, E) *S. lutea*, F) *S. aureus* and G) *V. paralyticum*.

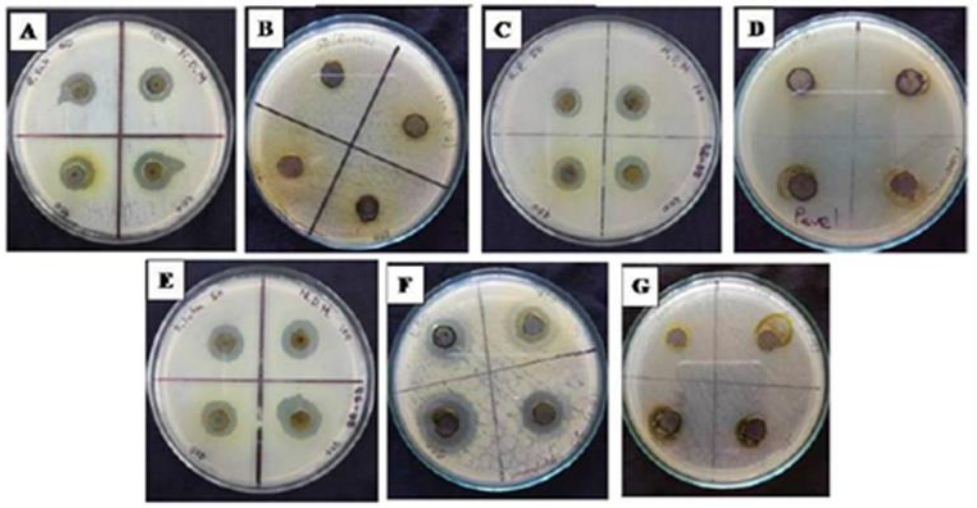

Figure2. Antibacterial activity of methanolic extract of *A. indica* (Pahari Neam) against A) *B. subtilis* , B) *E. coli*, C) *K. pneumoniae*, D) *P. aeruginosa*, E) *S. lutea*, F) *S. aureus* and G) *V. paralyticum*.

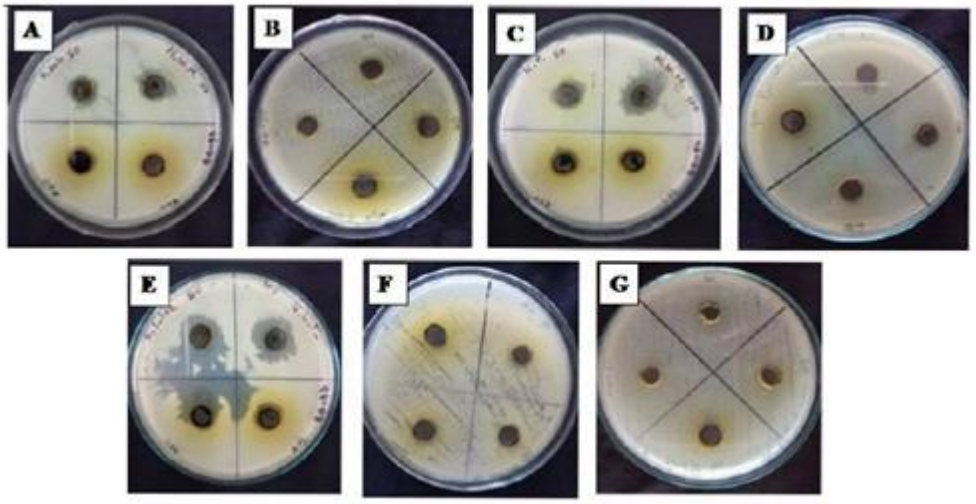

Figure3. Antibacterial activity of methanolic extract of *C. grandis* (Telakachu) against A) *B. subtilis* , B) *E coli*, C) *K. pneumoniae*, D) *P. aeruginosa*, E) *S. lutea*, F) *S. aureus* and G) *V. paralyticum*.

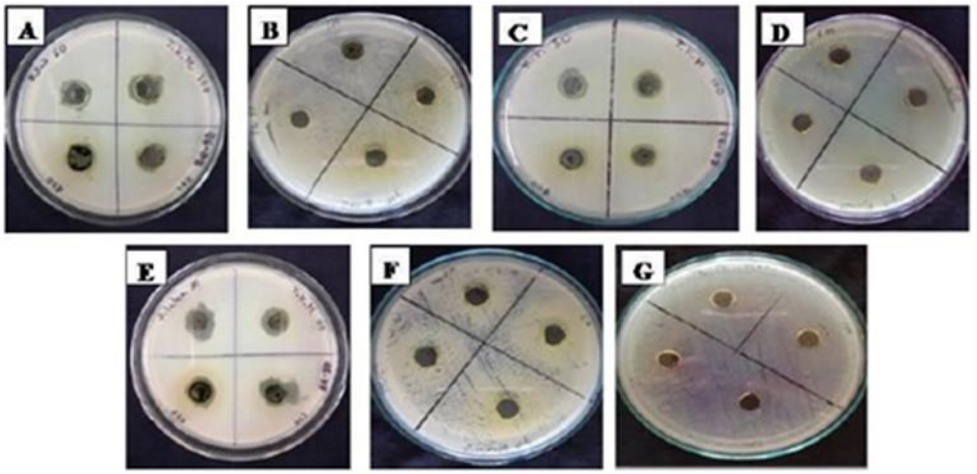

Figure4. Antibacterial activity of methanolic extract of *M. micrantha* against A) *B. subtilis* , B) *E. coli*, C) *K. pneumoniae*, D) *P. aeruginosa*, E) *S. lutea*, F) *S. aureus* and G) *V. paralyticum*

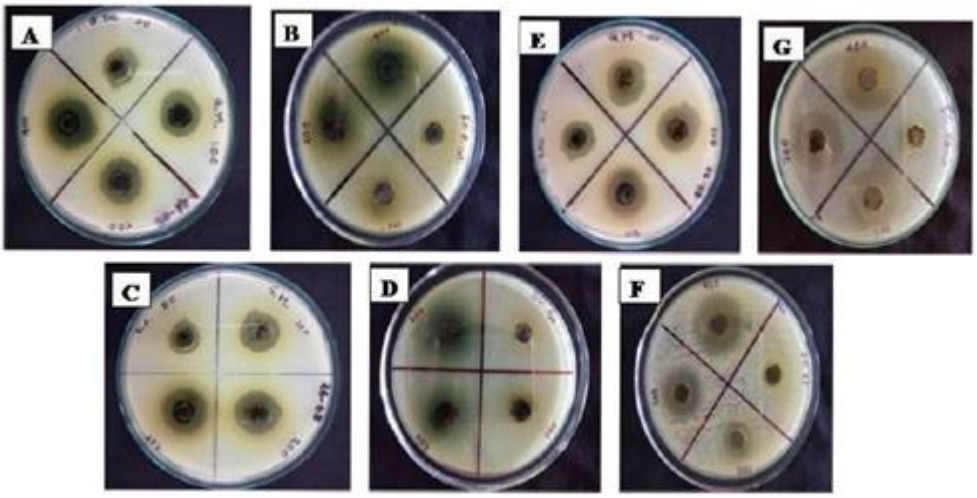

Figure5. Antibacterial activity of standard antibiotics against A) *B. subtilis* , B) *E. coli*, C) *K. pneumoniae*, D) *P. aeruginosa*, E) *S. lutea*, F) *S. aureus* and G) *V. paralyticum*