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Antimicrobial Activity of *Phyllanthus Emblica* and *Allium Sativum*: Comparative Analysis of Antimicrobial Action of Crude and Ethanolic Extract of These Natural Plant Products

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Abstract: *The antimicrobial effects of extracts of A. sativum and P. emblica, against pathogenic Staphylococcus aureus, Escherichia coli, Pseudomonas sp., Klebsiella sp, Salmonella typhi, Salmonella paratyphi, Salmonella sp., Bacillus subtilis, Bacillus cereus were investigated using the agar well and disc diffusion method. Aqueous extracts of A. sativum and P. emblica were more effective than ethanolic extarct producing larger zones of growth inhibition. All of natural products showed the MIC values ranged from 6.25–25.0µg/ml while the MBC values ranged from 12.5– 100.0µg/ml. This study is an indication that the A. sativum and P. emblica has the potentiality to use as a source for new broad spectrum oral antibiotics.*
Key words*: Antimicrobial activity, crude and ethanolic extract, MIC, MBC.*

I. Introduction

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide humankind with new remedies (Samuelsson, 2004). Many drugs used in contemporary medicine have been derived from plants and were originally discovered through the traditional use by indigenous people. The use of plants as medicines Isolation and characterization of pharmacologically active compounds from medicinal plants continue today. There has been resurgence in the consumption and demand for medicinal plants. These plants are finding use as pharmaceuticals, neutraceuticals, cosmetics and food supplements. Even as traditional source of medicines and they continue to play pivotal rule. The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia contains at least 25% drugs derived from plants. Many other are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing due to growing recognition of natural products, being non-toxic, having no side-effects, easily and developed countries available at affordable prices.

Allium sativum has been in used since ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton, 2002; Yeh. and Liu, 2001; Gardner *et a*l., 2003) and regular use of *A. sativum* may help to prevent cancer,to treat malaria, and to raise immunity. *A. sativum* has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella, Shigella* and *Staphylococcus aureus* (Teferi and Hahn 2002).Therapeutic use of *A. sativum* has been recognized as a potential medicinal value for thousands of years to different micro-organisms. For example, antifungal, antiviral, antibacterial antihelmantic, antiseptic and anti-inflammatory properties of *A. sativum* are well documented. Moreover, *A. sativum* extracts exhibited activity against both gram negative (*E. coli*, *Salmonella* spp. and *Citrobacter* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Kilabsella* sp.) and gram positive (*S. aureus, S. pneumonia,* Group A *Streptococcus* and *Bacillus anthrax*) all of which are causes of morbidity world wide. *Phyllanthus emblica* has been used for the treatment of several disorders such as the Scurvy, Cancer and Heart diseases. The important constituent of plant leaves have the anti-neutrophilic activity and anti-platelet properties in vitro. The extracts also posses several pharmacological properties like anti-viral(HIV, AIDS, HERPES VIRUS, CMV) antimutagenic, anti-allergic, anti-bacterial activities. The present study was designed to determine the antimicrobial activity of *A. sativum* and *P. emblica* and aslo focused on the comparison of only those commercial antibiotics.

II. Materials And Methods

Collection of the plants: The selected parts these plants were collected from Bishoroad and.Mohakhali, Dhaka; Monohardi, Narsingdi and gaibandha sodar, Gaibandha during the months of November to December, 2011.

Test microorganisms: *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* these organisms were collected from the Microbiology research laboraroty,

Department of Microbiology, Primeasia University of Dhaka. The pure of which was previously collected from the Microbiology Department of Dhaka University.

Pure Culture preparation: With the help of a inoculating lop, the test organisms were transferred from the pure culture to the agar slants in a laminar unit, the incubated satins were then incubated at 37ºC for 18-24 hours to ensure the growth of test organisms. This culture was used for sensitivity test.

Drying and Pulverization: The fresh plant products were first washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days. After complete drying, the entire portions were pulverized into a coarse powder with the help of a blender machine and were stored in an airtight container for further use.

Preparation of extracts:
Mechanical process Aqueous extract of *A. sativum* and *P. emblica* were prepared according to methods previously reported by Onyeagba et al. (2004). 100 g of fresh, washed *A. sativum* cloves was macerated in a sterile, ceramic mortar.The homogenate was then filtered off with a sterile, muslin cloth and used directly for the sensitivity test.To ensure aseptic conditions, sterile gloves and face masks were worn and the entire extraction was carried out in a media room of the Microbiology Laboratory, Primeasia University, Dhaka.
Ethanolic process The powdered 10gm powders were added with 100ml distiled ethanol in a conical flasks.then the solution was kept in room temperature for 24-72 hours.after that the homogenate was vaccume filtered with a sterial filter paper.then the extraction was kept in a sterial container and stored at -20 degree celcicus.

Standardization of inoculums: (Murray *et al*. 2007) A standard stock of the bacteria isolates were prepared by suspending a loop full of each microbial growth in about 10 ml of sterial saline . After incubation at 37°C for 12 h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about 1.4×10^7 cfu m/L.

Susceptibility test: Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Each Nutrient agar (NA) agar plate was uniformly seeded by means of sterile spreader dipped in the suspension and spreaded on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed (Murray *et al*. 2007). Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched in the NA agar with a sterile cork-borer. Approximately 100 µl of the extracts were dropped into each well which filled them respectively to fullness. The setup were allowed to stabilize for 3 h before being incubated at 37°C for 24 h as described previously by Shahidi (2004), Murray *et al*. (2007) and Aibinu *et al*. (2007).

Determination of the minimum inhibitory concentration (MIC) and MBC The MIC of the crude extract was determined using the doubling dilution method of Sahm and Washington (1990). Briefly, 1 ml of the reconstituted crude extract at a concentration of 100 mg/ml was added to 1 ml of nutrient broth. 1 ml of this extract concentration was transferred to another test tube and this dilution continued until an 7th test tube was reached, giving extract concentrations of 100, 50, 25, 12.5, 6.25, 3.17 and 1.58. mg/ml in different test tubes(v\v in case of aqueous extract) and (w|v in case of ethanolic extract). Then 100 µl of an 18 h culture of bacteria was inoculated into each of the test tubes and the contents horoughly mixed. The tubes were incubated at 37oC for 24 h. The 8th test tube contained 1 ml of pure solvent but no extract and served as a negative control. The test tube with the lowest concentration of the extract that did not show any detectable growth was taken as the MIC. Dilutions showing no visible growth for the MIC was sub-cultured and incubated at 37°C for 24h. The lowest concentration of the extracts yielding no growth on the NA plate was recorded as the MBC.

Test Antibiotic In our present study, the antibacterial activity of aqueous extracts and ethanol extract were investigated in compare with standard Tetracycline (30µg/disc), Cipproflaxacin (5µg/disc), Vancomycin (30µg/disc), Gentamycin (10µg/disc), Cephalothin (30µg/disc), Meropenam (10µg/disc) and Chloramphenicol (30µg/disc) antiibiotic agent a number of pathogenic Gram-positive and Gram-negative bacteria

III. Results

Aqueous and ethanolic extract of *A. sativum*, *P. emblica*, were tested (well method) for their antimicrobial activety against all of the selected microorganisms. In this antibacterial screening of crude extract were used at a concentration of 100 µg/well. Table 1A shows zone of inhibition of *A. sativum* and *P. emblica* (crude extract). It was observed that the aqueous extracts of *A. sativum* showed remarkable activity against all the tested organisms with the highest activity found on *Psudomonas* sp.(38mm zone diameter) at 100µg/well concentration. Zone of inhibition showed against A. *sativum* were satisfactory antimicrobial activity. Onyeagba

et al. (2006) worked on the same (*A. sativum*) and found no zone of inhibition against *A. sativum*. This is may be due to the species difference or the *A. sativum* difference in different biologic condition. Aqueous extract of *P. emblica* was also showed a good activity against all the tested organisms. De Britto *et al.* (2011) showed satisfactory activity of *P. emblica* against *Xanthomonas* sp., *Aeromonas* sp. and *Campestris hydrophila* sp., but in present study it was more effective result was found aginst all the selected microbes. On the other hand, the ethanol extract of *A. sativum* (Table 2)is also active against the test organisms with highest activity on *S. aureus* (17mm*)* at 100µg/disc, but Yusha'u *et* al (2008) observed that ethanol extract of *A. sativum* showed highest (51mm) activity on *E. coli* at 200µg/disc (Habib *et al*. (2007) reported sensitivity pattern of different microbes against chloroform extraction of *P. emblica* where no sensitivity was found whereas inpresent study, zone of inhibition was observed against all the tested organimsms. This difference of observation between Habib *et al* and present study may be due to species differences or ethanolic effect.

Determination of MIC and MBC It was observed that the MIC of *A. sativum* against *Salmonella sp., S. typhi, B. cereus* and *S. aureus* were 12.6 µg\ml whereas MIC against *Klebsiella sp.,* was *3.12 S. paratyphi,* MIC against *B. subtilis* and *Psudomonas* and *E. coli* were 6.25µg\ml.The MBC of *A. sativum* against *Salmonella sp.* and *S. paratyphi* were 100µg\ml , 25 µg\ml MBC was observed against *Klebsiella* sp. and *E.coli* and 50 µg\ml MBC was observed against all other organisms (Fig 1A).Whereas, the MIC of *A. sativum* (ethanolic extract) against *Klebsiella* sp. *Salmonella* sp. and *S. aureus* were 50.0µg\ml and *Psudomonas* sp. *S. typhi, B. cereus, B. subtilis* were 25.0µg\ml. MBC were found 100µg\ml against all microbes (Fig 1B). In Figure 2A, it was observed that the MIC of *P. emblica* against *Salmonella sp., S. typhi, B. cereus* were 12.6 µg\ml whereas MIC against *Klebsiella sp., S. paratyphi,*MIC against *B. subtilis* and *Psudomonas, S. aureus* and *E. coli* were 6.25µg\ml.The MBC of *P. emblica* against *Salmonella sp.* and *S. paratyphi* were 100µg\ml , 25 µg\ml MBC was observed against *E. coli* and 50 µg\ml MBC was observed against all other organisms. It was observed that the MIC of *P. emblica* (ethanolic extract)against *Salmonella* sp., *B. cereus B. subtilis* and *S. aureus* were 12.5µg\ml; whereas MIC against *S. typhi, Psudomonas* sp. and *Klebsiella* sp. were 25.0µg\ml. The MBC of *A. sativum* against *Salmonella* sp., *Klebsiella* sp. *Psudomonas* sp. and *S. paratyphi* were 100µg\ml and 50µg\ml MBC was observed against all other organisms (Fig 2B). Fani *et al.* (2007) studied on MIC of *A. sativum* against *S. mutans* ranged from 4-32 mg/mL which was slightly lower compared with our study where *A. sativum* (aqueous extract) showed MIC from 6.25 to 25µg\ml. This may be due to the species differences. Owhe-Ureghe *et al.* (2010) observed that the MIC of *A. sativum* against the test isolates ranged from 31.25 - 62.5 mg/mL whereas in present study, MIC of *A. sativum* ranges from 6.25-25.0 µg\ml in case of aqueous exrtracts.

Comparison with commercial antibiotics In case of *Klbesiella* sp., highest zone of inhibition was observed 38mm against *A. sativum* (aqueous). Clear zone of inhibition of 19mm was found in case of *P. emblica* (aqueous); whereas 29, 25 and 17 mm clear zone were observed against Tetracycline, Ciproflaxacin and Cholamphenicol, respectively. In case of *Psudomonas* sp. 39 and 21mm clear zones were showed against *A. sativum* (aqueous) and *P. emblica* (aqueous) respectively whereas clear zone by choramphenicol and ciprofloxacin were 23mm. At the time *of S. typhi* clear zones were 30 and 22mm against *A. sativum* (aqueous) and *P. emblica* (aqueous), respectively. 28 and 20 mm clear zones were showed against Cholamphenicol and Ciproflaxacin (Figure 3A). In case of *E.coli* clear zone of inhibition of 20 and 29mm was observed against *P. emblica* (aqueous) and *A. sativum* (aqueous), respectively; whereas zone of inhibition by Ciproflaxacin, Cefotaxime, Choramphenicol and Gentamicin were 30, 29, 26 and 28, respectively against *E.coli*. In case of *B. subtilis*, zone of inhibition 32mm was found against *A. sativum*, at the same time, zone of inhibition of 30 mm was observed against Ciproflaxacin. In case of *B. cereus A. sativum* (aqueous) and *P. emblica* (aqueous) showed 34 and 20mm zone of inhibition. Clear zone by Ciproflaxacin, Merupenam and Vancomycin were 30, 30 and 20mm, respectively (Figure 3B). Satisfactory results were observed only in case *A. sativum* and *P. emblica* (aqueous extract) as compared with commercial antibiotics. Sometimes *A. sativum* was better than the commercial one.

Extracts of P. *emblica*, *A. sativum* and showed that they are effective against all the tested pathogens *Psudomonas* sp., *S. aereus, Salmonella sp., S. typhi, S. paratyphi, B. cereus, B. subtilis, E. coli, S. aureus* in controlling their growth *in vitro* in culture condition. They all have a bacteristatic and bactericidal activity when tested *in vitro* using crude preparation. All of the plant products (Aqueous and ethanolic) *A. sativum* and *P. emblica* (aqueous extract)were best .they both could be able to inhibit the growth of all the sensitive microbes. Here the microbes we selected for our experiment can cause different types of diseases.As aqueous extracts of *A. sativum* and *P. emblica* are more sensitive to the microbes .Although in some cases *A. sativum* was more effective than commercial antibiotics like tetracycline and chloramphenicol. gallic and *P. emblica* may be used successfully for treating various diseases causing microbes agent *S. aureus., Salmonella, E. coli, S. aureus* etc. Further *in vivo* studies are necessary.

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Table 1 A: Determination of antimicrobial activity of *A. sativum* and *P. emblica* (Crude Extract)

Name of organisms	Zone of inhibition (mm)	
	A. sativum	***P. emblica***
Psudomonas sp.	38	20
S. typhi	28	22
S. aureus	24	24
E. coli	27	18
Klebsiella sp.	36	19
S. paratyphi	30	21
B. subtilis	28	20
Salmonella sp.	39	19
B. cereus	22	22

Table 1B: Determination of antimicrobial activity of *A. sativum* and *P. emblica* (Ethanolic Extract)

Name of organism	Zone of inhibition (mm)	
	A. sativum	***P. emblica***
Klebsiella sp.	11	15
B. subtilis	12	16
B. cereus	6	11
Psudomonas sp.	17	15
S .typhi	10	9
E. coli	0	0
S. paratyphi	0	9
S. aureus	17	10

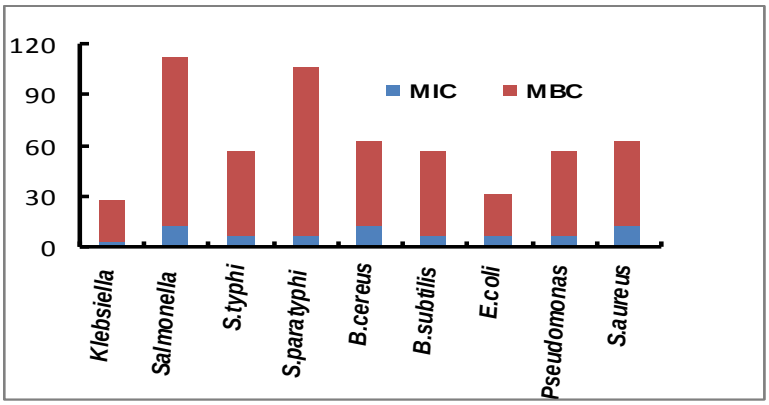

Figure1A- Determination of MIC and MBC of *A. sativum* (crude extract)

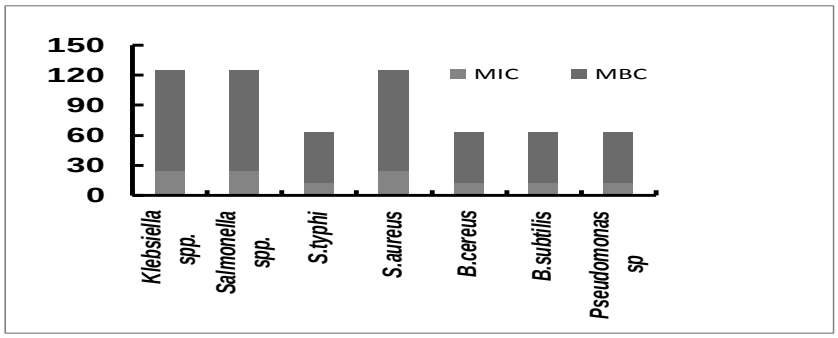

Figure1B- Determination of MIC and MBC of *A. sativum* (Ethanolic extract)

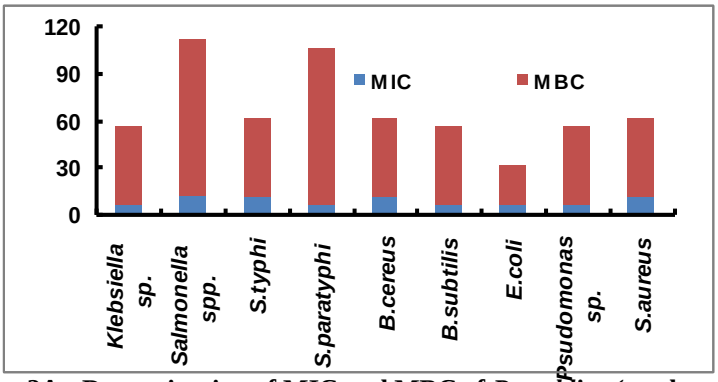

Figure 2A - Determination of MIC and MBC of *P. emblica* (crude extract)

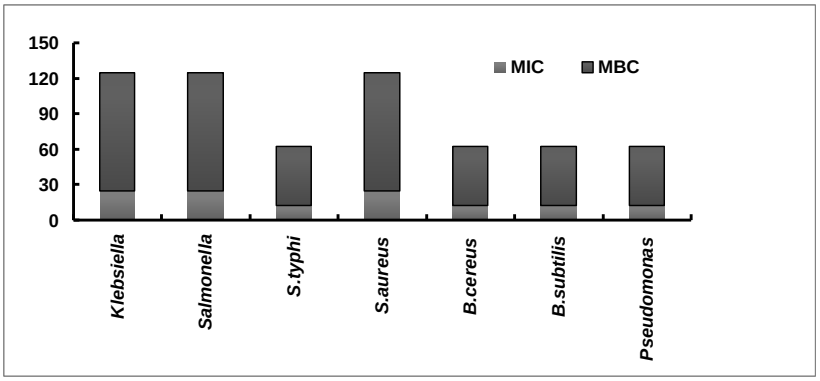

Figure 2B - Determination of MIC and MBC of *P. emblica* (Ethanolic extract)

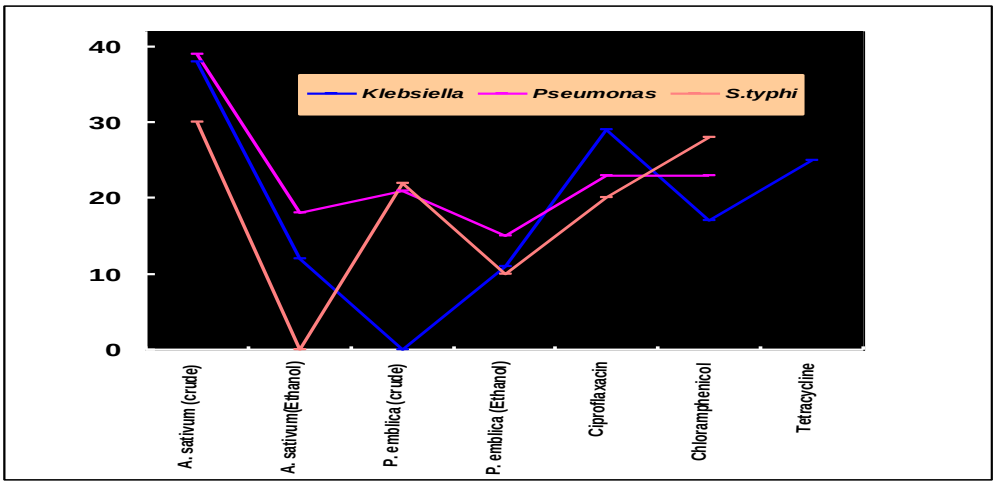

Fig. 3 A: Comparative analysis of A. sativum and P. emblica with commercial antibiotics

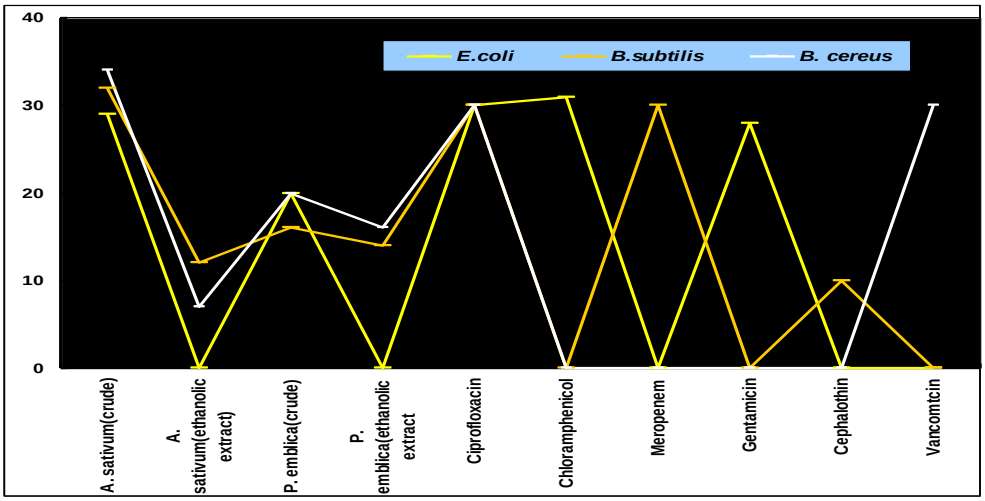

Fig. 3 B: Comparative analysis of A. sativum and P. emblica with commercial antibiotics