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# Synergistic Interactions of Chloroform Extract of Medicinal Plants with Antibiotics against Bacteria of Clinical Relevance

**Suman Kumari, Hitesh Kumar and Preeti Jain***

*Department of Biochemistry, Maharishi Dayanand University, Rohtak, Haryana India*

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## ABSTRACT

*Objective: To evaluate the synergistic effect of antimicrobial plant extracts from traditional medicinal plants on standard microorganism strains. Method: In the present study, eight plants (extracted with five different solvents) were screened for antibacterial activity against four gram negative and four gram positive strains by micro-broth dilution assay. Afterward, antimicrobial chloroform plant extracts were evaluated for synergistic effect with antibiotics against all the selected bacteria. Results: Chloroform extract of A. pungens, D muricata, S quitoense and G celosides exhibits antibacterial potential against all the bacterial strains. Almost all extracts shows synergistic effect with gram-positive bacteria while in case of gram-negative C coloynthis fruit and D muricata extracts shows synergistic interaction with antibiotics. Conclusion: The mixture of plant extract with antibiotics shows lower MIC. The plant extract do not exhibiting significant MIC were also found to show synergistic effect with antibiotics. It means plant extracts improves the activity of antibiotics. It can be inferred that the mixture of antibiotics and herbal remedies of these plant can be further evaluated to treat those infectious diseases that are caused by tested bacterial strains.*

**Keywords:** Antibacterial activity; Synergistic effect; Gram-positive bacteria; Gram-negative bacteria; Herbal extract

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## INTRODUCTION

Plants and herbs contribute to medicinal system since the early age of humankind and are still used throughout the world to treat pathogenic diseases and for health promotion. Plants are earliest and richest source of of bioactive compounds even then bacteria or fungi were also not used to extracts antibiotics. The discovery of antibiotics greatly improved the quality of healthcare system and human life in the nineteenth century. However, clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens [1,2]. It seems that pre-antibiotics era will return due to failure of antibiotics in prevention and control of various diseases. Plants still being substantial source of bioactive compounds significantly contribute commercially to drug preparation and form the basis of modern medicinal system [3]. Plants are reliable source to isolate bioactive compounds for direct use as drugs [4]. Plant based medicines showed few side effects, were cost effective and possessed better compatibility [5]. Earlier reports showed that plant metabolites possess positive interactions or synergistic effect with antibiotics and are often proved more effective than isolated antibiotics. Therefore, this study was designed to evaluate the antimicrobial potential and synergistic effect of plant extract with antibiotics. These plants are used in Ayurveda and traditional medicinal system for the treatment of various infections caused by pathogenic microorganisms. In the present study, *Alternathera pungens* (Amaranthaceae), *Citrullus colocynthis* (Cucurbitaceae), *Digera muricata* (Amaranthaceae), *Gomphrena celosioides* (Amaranthaceae), *Helianthus annus* (Asteraceae), *Ipomoea pestigirdis* (Convolvulaceae), *Leucas aspera* (Labiatae) and *Solanum quitoenes* (Solanaceae) plant materials were extracted with different solvents in increasing order of their polarity and were screened against gram negative and gram positive bacteria to find active plant extract possessing antimicrobial activity.

## MATERIALS AND METHODS

### Chemicals and Apparatus

Hexane, chloroform, acetone, methanol, sterile distilled water, dimethyl sulphoxide (Hi-media), HCl, ampicillin, nutrient broth, ethanol, resazurin dye, autoclave (Hicon), laminar flow (Metrex), incubator shaker (Remi), Halo DB 20 spectrophotometer (Dynamica), spinx vortex shaker (Tarsons), water bath (Hicon), centrifuge (Remi) and 96-well plates.

### Microorganism

Lyophilized culture of gram-positive bacteria (*Staphylococcus aureus* NDCC-109, *Bacillus cereus* NDCC-240 and *Bacillus subtilis* NDCC-215*)* and gram-negative bacteria (*Klebsiella pneumonia* NDCC-138, *Escherichia coli* NDCC-135, *Pseudomonas aeruainosa* NDCC-105 and *Salmonella typhi* NDCC-71) were obtained from National Dairy Research Institute, Karnal in September 2013. *Streptococcus pyogenes* MTCC-1076 was purchased from IMTECH, Chandigarh.

### Plant Material

Fresh materials of eight medicinal plants were collected from their natural habitat Rohtak, Haryana, India in September, 2012 to February, 2013. The *A. pungens* (whole plant), *C. colocynthis* (fruit & leaves), *D. muricata* (leaves), *G. celosioides* (whole plant), *H. annus* (leaves), *I. pestigirdis* (leaves), *L. aspera* (leaves), and *S. quitoenes* (leaves) were collected. Identification of the plants was done from Department of Botany, M. D. University, Rohtak and voucher specimen number are given in Table 1.

### Extraction

The properly dried plant materials were crushed and grinded to fine powder. For each plant part, 100 g of material was macerated three times for 72 h with five different solvents (100 ml each) in ascending order of polarity i.e., petroleum ether/hexane, chloroform, acetone, methanol and water. The combined extracts were filtered and solvents were evaporated to dryness in evaporatory rotator under reduced pressure below 50°C to yield crude extracts. The extracts were stored at -20°C until further use.

**Table 1: List of medicinal plants used under this study**

| Plant name | Voucher Sp. No. | Family name | Part used | Ayurvedic/traditional Use |
|---|---|---|---|---|
| *D. muricata (Lesua)* | 125/2012 | Amaranthaeceae | Leaves | Used for treatment of kidney stone & urinary tract disorder |
| *A. pungens (Khaki)* | 126/2012 | Amaranthaceae | Whole plant | Diuretic properties, gonorrhea |
| *G. celosioides (Prostrate globe- amaranth)* | 127/2012 | Amaranthaceae | Whole plant | Liver disease |
| *S. quitoense (Naranjilla)* | 128/2012 | Solanaceae | Fruits | To make beverages, also have nutritional value |
| *I. pestigridis (Panchpatia)* | 156/2013 | Convolvulaceae | Leaves | Treatment of skin disorder |
| *C. coloynthis (Bitter cucumber)* | 157/2013 | Cucurbitaceae | Fruit & Leaves | As most violent purgative drug, as energy source and as oilseeds |
| *H. annuus (Sunflower)* | 158/2013 | Asteraceae | leaves | Antioxidant, anti-inflammatory & diuretic properties |
| *L. aspera (Goma madhupati)* | 159/2013 | Labiatae | Flower & root | External application mostly for skin snake bite & wounds |

### Preparation of Inoculums

Using aseptic conditions, each bacterial culture were transferred to flasks containing 100 ml nutrient broth and placed in incubator for 15-18 hrs at 35°C. All the cultures were centrifuged at 4,000 rpm for 5 min. The supernatants were discarded and clean samples of bacteria were prepared. This step was repeated until the supernatant had become clear. The optical density of these bacterial suspensions was measured spectrophotometrically at 600 nm and serial dilutions were carried out with appropriate aseptic techniques until O. D. becomes 0.6. The required dilution factor was calculated and the dilution was carried out to obtain a concentration of $10^6$cells/$m^{-1}$ [6].

### *In vitro* Antibacterial Screening

The antibacterial activity of chloroform extracts were determined by the micro broth dilution assay in 96-well culture plates. Pour 100 µl of autoclaved nutrient broth to all the wells of culture plate. Then, 100 µl of test material was added to the first row of microtiter plate. Two-fold serial dilution was done throughout the column. 10 µl of 4 mg/ml resazurin solution was used as indicator. Finally, 10 µl of bacterial inoculum was added to each well. Proper positive and negative controls were kept for each experiment. The plates were incubated at 37°C and examined for change in colour of resazurin dye. Resazurin is violet-blue dye irreversibly reduced to the pink colour in presence of viable bacterial cell. The extracts were consider to be active if the wells appear

violet without any visible growth of bacteria and the result was expressed as Minimum Inhibitory Concentration (MIC) [7].

**Synergistic Effect of Plant Extracts with Antibiotics**

Synergistic effect of chloroform plant extracts with antibiotics was determined by the micro broth dilution assay as applied above for determination of MIC. In this assay, 50 µl plant extract+50 µl antibiotics (double the concentration of MIC value) was added in first well of 96-well plate instead of adding 100 µl of test material to check the synergistic action of both. Concentration of stock solution of plant extract/antibiotics were kept double of MIC as calculated above.

## RESULTS AND DISCUSSION

**Antibacterial Activity**

Chloroform extracts of all the plants were evaluated for their antimicrobial potential against eight bacteria by micro-broth dilution assay on 96-well plate. Minimum inhibitory concentrations (MIC) of plant extracts obtained against different bacteria are summarized in Table 2.

**Table 2: Antimicrobial activity of chloroform extract of selected plants (mg/ml)**

| Plant extract | BC | BS | SA | SP | EC | PA | ST | KP |
|---|---|---|---|---|---|---|---|---|
| *A.pungens* | 0.078 | 0.0097 | 0.0048 | 0.312 | 5 | 3.125 | 2.5 | 3.125 |
| *C. coloynthis* (L) | 0.625 | 0.156 | 0.039 | 1.25 | 2.5 | 3.125 | 5 | 1.56 |
| *C. coloynthis* (F) | - | 1.25 | 2.5 | 2.5 | 5 | 5 | 5 | 5 |
| *D. muricata* | 0.156 | 0.156 | 0.039 | 2.5 | 5 | 1.56 | 2.5 | 1.56 |
| *G. celosides* | 0.156 | 0.039 | 0.039 | 2.5 | 1.25 | 3.125 | 0.625 | 3.125 |
| *H. annus* | 0.625 | 0.039 | 0.078 | 0.156 | 5 | 5 | 5 | 3.125 |
| *I.pes-tigiris* | 0.625 | 0.312 | 0.0781 | 1.25 | 5 | 5 | 2.5 | 3.125 |
| *L. aspera* | 0.0195 | 0.039 | 0.078 | 0.156 | 2.5 | 5 | 1.25 | 3.125 |
| *S. quitoens* | 0.039 | 0.039 | 0.0195 | 0.625 | 2.5 | 1.56 | 1.25 | 1.56 |

Note: BC: *Bacillus cereus;* BS: *Bacillus subtilis;* SA: *Staphylococcus aureus*; SP: *Streptococcus pyogens;* EC: *Escherichia coli*; PA: *Pseudomonas aeruainosa;* ST: *Salmonella typhi;* KP: *Klebsiella pneumonia*

Ampicillin was used as positive control showing MIC within range 0.0097 to 0.156 mg/ml against different bacterial strains. All plant extracts showed different antimicrobial potential against different bacterial strains. *C. coloynthis* (Fruit) plant extracts were found to be least active among all the extracts. *A. pungens*, *C. Coloynthis*, *D. Muricata*, *G. celosides* and *S. quitoens* chloroform extracts exhibited considerable antibacterial potential in the range of 0.0048 to 3.125 mg/ml against all the eight bacterial strains (Table 2). *H. annus*, *I. pes-ti-giris* and *L. aspera* plant extracts were effective against gram-positive bacteria whereas weak antibacterial activity was reported against gram-negative bacteria in the range of 1.25 to 5.0 mg/ml. These plants were least active against *P. aeruainosa* whereas moderately active against *E. coli.* Overall, these plant extracts exhibit lower MICs against gram-positive bacteria in comparison to gram-negative bacteria. The compounds responsible for the antimicrobial activity of chloroform extracts were mainly non-polar in nature [8].

**Synergistic Effect of Antibiotics and Plant Extracts**

MIC of all the chloroform plant extracts in combination with ampicillin and streptomycin antibiotics are shown in Tables 3 and 4 respectively.

**Table 3: MIC of synergistic effect of chloroform plant extracts with antibiotics amphicillin against different bacteria (mg/ml). For this 50 µl plant extract + 50 µl ampicillin were added in first well instead of adding 100 µl antibiotics/plant extract. Concentration of stock solution of plant extract/antibiotic was kept double of MIC as observed by micro broth dilution assay**

| Extract | BC | BS | SA | SP | EC | PA | ST | KP |
|---|---|---|---|---|---|---|---|---|
| AP | *0.0047+0.0019 | *0.0047+ | 0.0048+0.015 | 0.078+0.062 | 2.5+0.062 | 1.56+0.062 | 1.25+0.031 | 1.56+0.031 |
| CC | *0.078+0.0039 | *0.312+0.015 | *0.0095+0.015 | 0.039+ | 2.5+0.062 | 1.56+0.062 | 2.5+0.031 | 0.78+0.031 |
| CCF | - | *0.625+0.0156 | *0.156+0.0039 | 0.125+0.0039 | *2.5+0.031 | *2.5+0.062 | *1.25+0.031 | *2.5+0.015 |
| DM | *0.0095+0.0039 | *0.0195+0.0078 | 0.078+0.125 | 0.125+0.0039 | *1.25+0.031 | *0.78+0.062 | *1.25+0.031 | *0.39+0.015 |
| GC | *0.0095+0.0019 | *0.019+0.015 | 0.0095+0.015 | *0.062+0.0039 | 0.625+0.062 | 1.56+0.062 | 0.312+0.03 | 0.78+0.015 |
| HA | *0.0047+0.0019 | *0.0195+0.015 | *0.019+0.015 | 0.625+0.125 | 2.5+0.062 | 5.0+0.062 | 2.5+0.031 | 1.56+0.031 |
| IP | *0.078+0.0039 | *0.156+0.015 | *0.0195+0.015 | *0.078+0.0039 | 5.0+0.062 | 5.0+0.062 | 1.25+0.031 | 1.56+0.031 |
| LA | *0.0011+0.0039 | *0.0195+0.015 | 0.078+0.0625 | 0.312+0.125 | 1.25+0.062 | 5.0+0.062 | 0.625+0.031 | 1.56+0.031 |
| SQ | *0.0047+0.0039 | *0.0095+0.0078 | *0.0023+0.0078 | *0.312+0.031 | 1.25+0.062 | 0.78+0.062 | 0.625+0.031 | 0.78+0.031 |

*Marked extract shows synergy, BC: *Bacillus cereus;* BC: *Bacillus subtilis;* SA: *Staphylococcus aureus*; SP: *Streptococcus pyogens;* EC: *Escherichia coli*; PA: *Pseudomonas aeruainosa;* ST: *Salmonella typhi;* KP: *Klebsiella pneumonia;* AP: *Alternathera pungens*; CC: *Citrullus colocynthis*; CCF: *Citrullus colocynthis* fruit; DM: *Digera muricata*; GC: *Gomphrena celosioides;* HA: *Helianthus annus;* IP: *Ipomoea pestigirdis*; LA: *Leucas aspera* and SQ: *Solanum quitoene*

**Table 4: MIC of synergistic effect of chloroform plant extracts with antibiotics streptomycin against different bacteria (mg/ml). For this 50 µl plant extract + 50 µl streptomycin were added in first well instead of adding 100 µl antibiotics/plant extract. Concentration of stock solution of plant extract/antibiotic was kept double of MIC as observed by micro broth dilution assay**

| Extract | BC | BS | SA | SP | EC | PA | ST | KP |
|---|---|---|---|---|---|---|---|---|
| AP | *0.0095+ 0.0039 | *0.0047+0.031 | *0.0023+0.031 | *0.039+0.0078 | 2.5+0.125 | 1.56+0.125 | 1.25+0.062 | 1.56+0.062 |
| CC | *0.156+0.0078 | *0.0195+0.0078 | *0.0047+0.0078 | *0.156+0.0078 | 2.5+0.125 | 1.56+0.125 | 2.5+0.062 | 0.78+0.062 |
| CCF | - | *0.312+0.0156 | *0.312+0.0078 | *0.625+0.0156 | *2.5+0.0625 | *2.5+0.062 | *1.25+0.031 | *2.5+0.062 |
| DM | *0.0195+0.0039 | *0.0195+0.0078 | *0.0095+0.0156 | *0.312+0.0078 | *1.25+0.0625 | *0.39+0.062 | *0.78+0.031 | *0.39+0.062 |
| GC | *0.0195+0.0039 | *0.0047+0.0078 | *0.0047+0.0078 | *0.625+0.0156 | 0.625+0.125 | 1.56+0.125 | 0.312+0.062 | 1.56+0.062 |
| HA | *0.156+0.0078 | *0.0047+0.0078 | *0.0095+0.0078 | *0.0195+0.0078 | 2.5+0.125 | 5.0+0.125 | 2.5+0.062 | 1.56+0.062 |
| IP | *0.078+0.0039 | *0.039+0.0078 | *0.0095+0.0078 | *0.312+0.0156 | 5.0+0.125 | 5.0+0.125 | 1.25+0.062 | 1.56+0.062 |
| LA | *0.0095+0.0039 | *0.0047+0.0078 | *0.039+0.0312 | *0.0195+0.0078 | 1.25+0.125 | 5.0+0.125 | 0.625+0.062 | 1.56+0.062 |
| SQ | *0.0047+0.0039 | *0.0047+0.0078 | *0.0023+0.0078 | *0.078+0.0078 | 1.25+0.125 | 0.78+0.125 | 0.625+0.062 | 0.78+0.062 |

*Marked extract shows synergy, BC: *Bacillus cereus;* BC: *Bacillus subtilis;* SA: *Staphylococcus aureus*; SP- *Steptococcus pyogens;* EC: *Escherichia coli*; PA: *Pseudomonas aeruainosa;* ST: *Salmonella typhi,* KP: *Klebsiella pneumonia;* AP: *Alternathera pungens*; CC: *Citrullus colocynthis*; CCF: *Citrullus colocynthis* fruit; DM: *Digera muricata*; GC: *Gomphrena celosioides;* HA: *Helianthus annus;* IP: *Ipomoea pestigirdis*; LA: *Leucas aspera;* SQ: *Solanum quitoenes*

Most of the plant extracts and their combinations with antibiotics were found to be more active than the extract and antibiotics alone. Antibiotics (ampicillin and streptomycin) exhibited MIC in the range 0.0097 to 0.156 mg/ml against different bacterial strains. It was found that streptomycin antibiotic shows more synergistic potential with these extracts than ampicillin. All the gram positive bacteria shows decreased MIC in combination with streptomycin whereas ampicillin was not found to be synergistic with *A. punguns*, *C. coloynthis* fruit, *D. muricata*, *G. Celosides*, *H. annuus* and *L. aspera* extracts. *I. pestigiris* and *S. quitoens* were found to show maximum synergistic effect against gram positive bacteria. In case of gram negative bacteria, only *C. coloynthis* fruit and *D. muricata* extracts exhibited positive interaction in combination with both antibiotics against all the strains. It is well known that many herbal extracts possess antibacterial activities. However, their potential alone is lower than antibiotics. Secondly, bacterial strains especially gram negative bacteria can easily develop resistance against antibiotics. Thus, the choice of effective and safe drug to be used against these bacteria is shrinking day by day. Therefore, these plant extracts can be searched to develop alternative or combination agent used to treat infectious disease. Overall results indicate that the best antimicrobial compound may not show the best synergy or *vice-versa*. Among all the plants *C. coloynthis* (F) and *D. muricata* extracts were most effective in synergy in case of gram-negative bacteria. In case of gram-positive considerable synergy was observed in *S. quitoense* and *I. pestigridis* extract. In conclusion, *S. quitoense*, *I. pestigirdis*, *C. colocynthis* and *D. muricata* seems to contain compounds that inhibit the growth of bacteria as well as shows synergy with antibiotics.

## CONCLUSION

It is well known that many herbal extracts possess antibacterial activities and shows positive interaction with antibiotics to kill bacteria. The plant extract having significant MIC will not necessarily shows synergistic effect with antibiotics. In the present time, bacterial strains, especially gram negative bacteria can easily develop resistance against antibiotics. The compounds from natural products including medicinal plants that show synergy with antibiotics will be helpful in the treatment of infection. Therefore, attention is needed to develop alternative or combination agent. Further exploration of these plant extracts for isolation of active compounds may be considered, which can be further used as therapeutic agents to control the antibiotic resistant in microbes.

## REFERENCES

[1] A Bartoloni; L Pallecchi; H Rodríguez; C Fernandez; A Mantella; F Bartalesi; M Strohmeyer; C Kristiansson; E Gotuzzo; F Paradisi; GM Rossolini. *Int J Antimicrob Agents*. **2009**, 33, 125-129.
[2] HK Allen; J Donato; HH Wang; KA Cloud-Hansen; J Davies; J Handelsman. *Nature Rev Microbiol*. **2010**, 8, 251-259.
[3] MM Cowan. *Clin Microbiol Rev*. **1999**, 12, 564-582.
[4] MF Balandrin; AD Kinghorn; NR Farnsworth. In ACS symposium series (USA) **1993**.
[5] AD Kinghorn; MF Balandrin. *American Chem Society (ACS).* **1993**.
[6] R Dabur; A Gupta; TK Mandal; DD Singh; V Bajpai; GS Lavekar. *Afr J.Tradit Complement Altern Med*. **2008**, 4, 313-318.
[7] M Khond; Bhosale; T Arif; TK Mandal. MM Padhi; R Dabur. *Middle East J Sci Res*. **2009**, 4, 271-278.
[8] P Masoko; JN Eloff. *Afr J Biotechnol*. **2005**, 4, 1425-1431.