Research Article
ISSN: 0974-6943

*Shweta M. Bhiwankar et al. / Journal of Pharmacy Research 2015,9(11),606-611*
Available online through
www.jprsolutions.info

# Study on *Acacia polyacantha* for Controlling the Multi Drug Resistance in *Enterobacteriaceae*

**Shweta M. Bhiwankar[1*], Ashok V. Gomashe[1] and Vijay N. Charde[2]**
[1]Department of Microbiology, Shri Shivaji Science College, Congress Nagar, Nagpur (Maharashtra), India.440009
[2]Department of Microbiology, Taywade College (Arts, Commerce and Science),
Mahadula-Koradi, Dist. Nagpur (Maharashtra), India.440009

**Received on:09-07-2015; Revised on: 22-08-2015; Accepted on: 27-09-2015**

## ABSTRACT

**Background:** The study carried out on the clinical samples possessing human bacterial pathogens possess drug resistance under *in vitro* conditions and further tested for the synergistic activity of *Acacia polyacantha* and antibiotics for better control. **Aims and Objectives:** Clinically isolated bacterial species were tested for their susceptibility against the thirty four antibiotics and determined for the antibiotic resistance using Kirby-Bauer method. Further several extracts of *A. polyacantha* were tested under synergistic study to select the best scored plant extract inhibiting maximum strains especially of *E. coli* and *Proteus* species in presence of antibiotics. **Results and Discussion**: Reduced zone of inhibition highlighted the present antibiotic resistance. In contrast, synergistic action of *A. polyacantha* methanolic extract of bark and leaf proved best in increasing the susceptibility in strains which showed prior resistance to antibiotics. **Conclusion**: Bacterial pathogens increasing in resistance and according to study combinational therapy may avert the cause of resistance if used carefully as evident with *A. polyacantha* extracts which improved the susceptibility even in those antibiotics which have showed complete resistance in *E. coli* and *Proteus* species.

**KEYWORDS:** *Acacia polyacantha*; Clinical sample; Drug resistance; Enterobacteriaceae.

## INTRODUCTION

As per definition, Enterobacteriaceae comprises of *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Providencia*, and *Serratia* species.[1] Enterobacteriaceae are inhabitants of the intestinal flora and important pathogens in both nosocomial and community settings. Enterobacteriaceae distributes easily between humans by physical contact as well as by contaminated food and water; they also acquire genetic material through horizontal gene transfer, mostly mediated by plasmids and transposons.[2,3] Resultant, enterobacteriaceae family members are the leading cause of bloodstream infections, urinary tract infections (UTIs), various intra-abdominal infections and hospital acquired pneumonias. The prevalence of extended-spectrum beta-lactamases (ESBLs) producing enterobacteriaceae made carbapenem, a broad-spectrum antimicrobial agent became a preferred drug in the treatment of multi-drug-resistant (MDR) enterobacteriaceae. In recent years, several reports about the emergence of even carbapenem resistant *Enterobacteriaceae* (CRE) become an alarming threat to public health. In any epidemic area, the prevalence of CRE varies between 24.7% and 29.8%[4,5] puts up a serious question in front of the medical treatments. The emergence of CRE is a real threat to patients, mainly to those who are suffering, with various underlying diseases, medical interventions or complex infections.[6]

***Corresponding author.**
***Shweta M. Bhiwankar***
***Department of Microbiology,***
***Shri Shivaji Science College,***
***Congress Nagar, Nagpur***
***(Maharashtra), India.440009***

Since many years, plants have been a precious source of natural products for maintaining human health. The use of plant compounds for pharmaceutical purposes has gradually increased. As per World Health Organization[7] medicinal plants would be the finest source to obtain herbal drugs. About 80% population in developed countries uses traditional medicine, which has compounds derived from medicinal plants. Therefore, medicinally important plants should be investigated to better understand their properties, safety and efficiency.[8] The antimicrobial properties of compounds obtained from *Parthenum argentatum* against *Candida albicans*, *Torulopsis*, *Hansemula*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was detected.[9,10] Likewise, antimicrobial activity from *Mikania triangularis*, known as "thin leaf guaco", was tested against five genera of bacteria and it showed activity against *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*.[11] Present study investigated the synergistic action of *A. polyacantha* extract and antibiotics against resistant Enterobacteriaceae to find out the potential of combinational therapy.

## MATERIALS AND METHODS

### Clinical Sample Collection

Human clinical samples of patients hospitalized in clinics of Nagpur with different ailments such as surgical wound, pneumonia, urinary tract infection, bacteraemia and respiratory infections have been considered under the study were sampled in sterile sampling bottles and labeled.

**Isolation of Enterobacteriaceae**

The clinical samples enriched on nutrient broth were further inoculated on the differential as well as selective medias such as Macconkey agar, Eosin Methylene Blue agar (EMB agar), Salmonella and Shigella agar (SS agar), Nutrient agar (NA), Bismuth and Sulfite agar (BSA), for the particular isolation of *Escherichia* sp., Enterobacter sp., *Klebsiella* sp., *Proteus* sp., *Shigella* sp., and *Salmonella* sp.

**Antibiotic Susceptibility Test**

The bacterial species identified as *Escherichia* sp., *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Shigella* sp., and *Salmonella* sp. were investigated for the susceptibility against the thirty four drugs using Kirby-Bauer method highlighted in Table 1.

**Table 1: Standard antibiotic zone size interpretative chart as per CLSI activity on Enterobacteriacae**

| Sr.No. | Antibiotics | Symbol | Resistant | Intermediate | Sensitive |
|---|---|---|---|---|---|
| 1 | Trimethoprim | TR | 10 | 11-15 | 16 |
| 2 | Tigecycline | TGC | 10 | 11-15 | 16 |
| 3 | Ofloxacin | OF | 12 | 13-15 | 16 |
| 4 | Kanamycin | K | 13 | 14-17 | 18 |
| 5 | Rifampicin | RIF | 17 | 18-20 | 21 |
| 6 | Sulphafurazole | SF | 12 | 13-16 | 17 |
| 7 | Cefepime | CPM | 14 | 15-17 | 18 |
| 8 | Cefuroxime | CXM | 14 | 15-17 | 18 |
| 9 | Chloramphenicol | C30 | 12 | 13-17 | 18 |
| 10 | Cefazolin | CZ | 14 | 15-17 | 18 |
| 11 | Nalidixic acid | NA | 13 | 14-18 | 19 |
| 12 | Co-Trimoxazole | COT | 10 | 11-15 | 16 |
| 13 | Nitrofurantoin | NIT | 14 | 15-16 | 17 |
| 14 | Minocycline | MI | 14 | 15-18 | 19 |
| 15 | Tobramycin | TOB | 12 | 13-14 | 15 |
| 16 | Neomycin | N | 12 | 13-16 | 17 |
| 17 | Amoxyclav | AMC | 13 | 14-17 | 18 |
| 18 | Cefixime | CFM | 15 | 16-18 | 19 |
| 19 | Colistin | CL | 10 | - | 11 |
| 20 | Ampicillin | AMP | 13 | 14-16 | 17 |
| 21 | Carbenicillin | CB | 19 | 20-22 | 23 |
| 22 | Tetracycline | TE | 14 | 15-18 | 19 |
| 23 | Ciprofloxacin | CIP | 15 | 16-20 | 21 |
| 24 | Imipenem | IPM | 13 | 14-15 | 16 |
| 25 | Netillin | NET | 12 | 13-14 | 15 |
| 26 | Ceftriaxone | CTR | 13 | 14-20 | 21 |
| 27 | Ceftazidime | CAZ | 14 | 15-17 | 18 |
| 28 | Meropenem | MRP | 13 | 14-15 | 16 |
| 29 | Cefotaxime | CTX | 14 | 15-22 | 23 |
| 30 | Gentamicin | GEN | 12 | 13-14 | 15 |
| 31 | Norfloxacin | NX | 12 | 13)-16 | 17 |
| 32 | Amikacin | AK | 14 | 15-16 | 17 |
| 33 | Cefoperazone | CPZ | 15 | 16-20 | 21 |
| 34 | Piperacillin/ Tazobactam | PIT | 17 | 18-20 | 21 |

**Plant Extracts Preparation**

In an order to control the increasing resistance against antibiotics, combined therapy of Ayurveda and Allopathic treatment proved to be effective against Enterobacteriaceae. In a view plant, *Acacia polyacantha* leaves and bark were air dried and powdered. About 25g were used in extraction process by suspending into 100 ml of polar and nonpolar solvents such as petroleum ether, chloroform, acetone, methanol and water to obtain the plant extracts, when treated under hot and cold extraction protocols using soxhlet apparatus and maceration process respectively.

For hot extraction, weighed 25 g dry plant powder filled in the Soxhlet thimble and refluxed with 100 ml of given solvent for 24 hours. Solvent were allowed to pass through in a sequence with increasing polarity such as petroleum ether followed by, chloroform, acetone, methanol and lastly by water. Before every solvent extraction, the material inside of the thimble was exposed to air and allowed complete evaporation of earlier solvent which has got absorbed in plant material. After 24 hrs of reaction, accumulated 55-60ml of solvent was recovered from round bottom flask. The solvent then exposed to evaporation at room temperature, until highly concentrated extract remained in the flask. Obtained concentrate was then diluted by adding the 50 ml of same extraction solvent and stored in a refrigerator till use.

In Cold extraction, weighed 25 g of dry plant powder were kept suspended in nylon cloth (400 mesh size) in 100 ml of aforementioned solvents in 250 ml stoppered conical flask. The flask kept rotary at 150 rpm in B.O.D incubator preset at $25^0$C for 24 hours of incubation. During cold extraction, same solvent based extraction, concentration and dilution protocol was followed as mentioned in hot extraction.

Based on the extractions, in total 20 different extracts were prepared and they were abbreviated such as:

- Hot Acacia Bark Petroleum ether extract (HABPE),
- Hot Acacia Bark Chloroform extract (HABCL),
- Hot Acacia Bark Acetone extract (HABAT),
- Hot Acacia Bark Methanol extract (HABME),
- Hot Acacia Bark Water extract (HABW),
- Hot Acacia Leaf Petroleum ether extract (HALPE),
- Hot Acacia Leaf Chloroform extract (HALCL),
- Hot Acacia Leaf Acetone extract (HALAT),
- Hot Acacia Leaf Methanol extract (HALME),
- Hot Acacia Leaf Water extract (HALW),
- Cold Acacia Bark Petroleum ether extract (CABPE),
- Cold Acacia Bark Chloroform extract (CABCL),
- Cold Acacia Bark Acetone extract (CABAT),
- Cold Acacia Bark Methanol extract (CABME),
- Cold Acacia Bark Water extract (CABW) ,

- Cold Acacia Leaf Petroleum ether extract (CALPE),
- Cold Acacia Leaf Chloroform extract (CALCL),
- Cold Acacia Leaf Acetone extract (CALAT),
- Cold Acacia Leaf Methanol extract (CALME),
- Cold Acacia Leaf Water extract (CALW).

**Inoculum development**

A loopful of culture from slants was inoculated into the screw cap tube containing 5ml sterile nutrient broth and incubated at 37°C for 24hrs. Again loopful of culture from same broth was transferred to fresh 5ml of sterile nutrient broth and incubated at 37°C for 6-8 hrs. Turbidity was adjusted according to 0.5 McFarland standards which were then used as inoculums which correspond to size of $1.5\times10^8$ CFU/ml.

**Synergistic Assay of Plant Extract and Antibiotics**

Based on the antibiotic resistance results, bacterial species shown more than 75% (25 drugs) antibiotic resistance were considered further in the synergistic control. Pipetted 100 µl of plant extract mixed with molten Hi-sensitivity test agar (15 ml) set at 50-55°C and then plated. Resistant isolates were inoculated at 1.5 x $10^6$ CFU/ml as per MacFarland scale and allowed to soak for 10 minutes. The antibiotic discs were placed and plates were firstly transferred for one hour in a refrigerator for uniform extract diffusion and then to an incubator preset at 37°C for 24 hours incubation. After incubation, the comparative change in the zone of inhibition recorded along with untreated group where extract was not added in media and analyzed for the extract based effective inhibition for each drug.

**RESULTS**

**Prevalence of Bacterial species**

The 525 clinical samples collected from hospitals were processed for the isolation of available bacterial species. After analysis, it has been recorded that a typical pattern of bacterial flora was available in each type of samples and those have been represented in Table 2.

**Table 2: Number of bacterial species isolated from given sources**

| Samples/Species | *E. coli* | *Klebsiella sp.* | *Proteus sp.* |
|---|---|---|---|
| Urine | 81 | 34 | 17 |
| Blood | 0 | 2 | 0 |
| Sputum | 3 | 4 | 0 |
| Pus | 5 | 0 | 4 |
| Stool | 5 | 0 | 5 |

| Samples/Species | *Shigella sp.* | *Salmonella sp.* | *Enterobacter sp.* |
|---|---|---|---|
| Urine | 0 | 0 | 10 |
| Blood | 1 | 11 | 0 |
| Sputum | 9 | 0 | 0 |
| Pus | 0 | 0 | 0 |
| Stool | 0 | 0 | 0 |

**Antibiotic Resistance in Isolates**

Based on the Kirby-Bauer method, clinical isolates showed increased drug resistance as calculated in percentage (%). The percentage resistance was divided into four parts and used further to grouping the isolates as given below (Table 3):

**Table 3: Grouping of bacterial isolates as per percent Antibiotic resistance**

| Culture | Groups | | | |
|---|---|---|---|---|
| | 1-25% | 26-50% | 51-75% | 76-100% |
| *E. coli* | 3 | 18 | 44 | 13 |
| *Klebsiella sp.* | 8 | 9 | 9 | 10 |
| *Proteus sp.* | 9 | 10 | 2 | 5 |
| *Enterobacter sp.* | 3 | 5 | 1 | 1 |
| *Salmonella sp.* | 5 | 6 | 0 | 0 |
| *Shigella sp.* | 1 | 6 | 3 | 0 |

In 78 *E. coli* strains existing antibiotic resistant was recorded. Based on the results, the percent resistance varies with each *E. coli* strain with the minimum resistance of 14.7% to the maxima of 91.17%. These 78 strains were further grouped where, three isolates shown 1-25%; eighteen isolates shown 26-50%; forty four isolates shown 51-75% and thirteen isolates have shown 76-100% resistance pattern showed the trend towards maximum antibiotic resistance (Table 3).

In 36 *Klebsiella sp*., a percent resistance minimum was 5.88% and surprisingly maxima up to 100% for the thirty four antibiotics tested. In strain grouping, eight isolates shown 1-25%; nine isolates with 26-50%; nine isolates with 51-75% and ten isolates with 76-100% antibiotic resistance pattern. In *Klebsiella* sp. alone one strain showed 100% antibiotic resistance i.e. to all 34 antibiotics which is posing concern in front of the public health (Table 3).

In 26 *Proteus sp*., a minimum percent resistance recorded to 8.82% to the maximum of 91.17% for the antibiotics. In strain grouping, nine isolates shown 1-25%; ten isolates shown 26-50%; two isolates shown 51-75% and five isolates shown 76-100% antibiotic resistance pattern (Table 3).

In 10 *Enterobacter sp*., a minimum percent resistance was 8.82% to the maximum of 79.4% for the antibiotics. In a strain grouping, three isolates shown 1-25%; five isolates shown 26-50%; one isolates shown 51-75% and one isolates classified in 76-100% resistance group (Table 3).

In 10 *Shigella sp*., a minimum resistance percent recorded was 8.82%, with the maximum of 49.99% for the tested antibiotics. In strain grouping, one isolate shown 1-25%; six isolates shown 26-50%; three isolates shown 51-75% and none isolates with 76-100% resistance (Table 3).

In 11 *Salmonella* sp., five isolates shown 1-25%; six isolates shown 51-75% and no isolates were classified in 51-75% and 76-100% resistance (Table 3).

**Synergistic action of Antibiotics and Plant extracts**

Even though study investigated the antibiotic resistance in six species mentioned earlier; synergistic activity of antibiotics and plant extracts was investigated with *E. coli* and *Proteus* species only as given below:

***E. coli***

The *E. coli* isolates possessing antibiotic resistant have shown the increased susceptibility with all the extracts when tested in combination with antibiotics. Prominently methanolic extracts of both hot and cold preparations of *A. polyacantha* leaves and bark produced maximum zone of inhibition in combinational testing. Comparatively, hot methanolic extracts of bark scored maximum zone of inhibition with an average of 25 ± 7 mm and doubles the inhibition compared to antibiotics treatment recorded as 15 ± 7 mm as highlighted in reference Table 4.

Additionally, methanolic hot extract of *A. polyacantha* bark also showed increased zone of inhibitions with all antibiotics against *E. coli* which was prior showing resistance. This indicated that available active phyto-chemicals in the bark methanolic extracts probably controlling the resistance and resultant inhibited the *E. coli* growth in presence of antibiotics.

The overall control by each plant extract with antibiotics was also calculated by sum of all the zone of inhibition obtained in each extract group. On that basis, the decreasing inhibition capabilities were recorded with *A. polyacantha* bark extracts as follows:
HABME>CABME>HABPE>HABCL>HABAT>CABAT>CABW> CABPE>CABCL>HABOCL>HABOPE> Standard.

Also, the leaf extract of *A. polyacantha* has shown similar trend up to certain extend and the overall decreasing inhibition with extracts was highlighted as follows:
HALME> CALME>HALPE>HALCL> CALAT>HALAT> CALPE> CALW >CALCL> HALW>HALAT>HALOPE> Standard.

Apart from methanolic extracts, other extracts have also shown the potent inhibition capability against *E. coli* as it was evident from calculated increased cumulative inhibition recorded in millimeter (mm), where HALME and HABME extracts scored best in overall sum of zone of inhibition when compared with other low scored groups.

Based on the group analysis, in bark the number of times highest zone of inhibition obtained in every extract group was counted and from that, it was apparent that with the given antibiotics, HABME scored highest (271 times) followed by CABME (51 times). In leaves extract also, HALME scored (156 times) highest followed by CALME (78 times).

**Table 4 : Representative synergistic activity of Bark extract along with Antibiotics showing increasing growth inhibition against *E. coli U13I1***

| Antibiotic | PIT | COT | T | CTX | K | MI | AK | CIP | TR | OF | RIF | NX | *E. coli* U13I1 CPZ | NA | CFM | GEN | CPM | TE | IPM | NET | CXM | CZ | AMP | CTR | MRP | CB | AMC | C | Total Zone of inhibition (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Extract↓ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Standard** | NI | NI | 13 | NI | NI | 11 | NI | 10 | NI | 10 | NI | NI | NI | NI | NI | NI | NI | NI | 10 | 15 | NI | NI | NI | NI | 10 | NI | NI | 10 | **89** |
| **CABPE** | 12 | NI | 19 | NI | 22 | 20 | 21 | 24 | NI | 27 | 12 | 23 | 17 | 19 | NI | 19 | NI | 24 | 37 | 16 | 18 | NI | NI | NI | **28** | NI | NI | 26 | **384** |
| **CABCL** | 12 | 16 | 18 | 15 | 18 | 22 | 19 | 27 | NI | 18 | 12 | 20 | 17 | 18 | NI | 20 | NI | 25 | 38 | 14 | 19 | NI | NI | 11 | 22 | NI | NI | 25 | **426** |
| **CABAT** | 11 | 13 | 17 | 10 | **24** | 21 | 17 | 25 | NI | 21 | **17** | **26** | 17 | 17 | NI | 20 | NI | 21 | 39 | 19 | 19 | 14 | NI | 12 | 22 | NI | NI | **29** | **454** |
| **CABME** | **14** | 11 | 17 | 14 | 20 | 22 | 22 | 27 | NI | 24 | 15 | **26** | NI | 15 | NI | 18 | NI | 24 | 36 | 19 | 18 | NI | NI | **14** | 25 | NI | NI | 28 | **431** |
| **CABW** | NI | NI | 17 | 13 | 20 | 19 | 20 | 27 | NI | 24 | 13 | 25 | 16 | 19 | **20** | NI | NI | 25 | **40** | **25** | **24** | NI | NI | NI | 24 | NI | **11** | 22 | **423** |
| **HABPE** | NI | 16 | 20 | 13 | 22 | 23 | 21 | **30** | NI | 22 | 12 | 23 | 15 | 17 | NI | **21** | NI | 24 | **40** | 15 | 17 | NI | NI | 10 | 25 | NI | NI | 25 | **433** |
| **HABOPE** | 12 | 14 | 18 | 16 | 15 | 17 | 17 | 24 | **10** | 23 | 15 | 24 | **20** | 17 | NI | 21 | **29** | 26 | 33 | 22 | NI | NI | NI | 11 | 18 | NI | NI | 28 | **413** |
| **HABCL** | 12 | **18** | 21 | 16 | 21 | **23** | 21 | 28 | **10** | **28** | 16 | 24 | 17 | **21** | NI | 20 | 25 | 25 | 33 | 23 | 14 | NI | NI | 11 | 22 | NI | NI | 28 | **474** |
| **HABOCL** | NI | 17 | 18 | **17** | 20 | 19 | 18 | 25 | NI | 24 | 13 | 21 | 17 | 16 | NI | 17 | NI | 21 | 34 | 17 | 19 | 14 | NI | **14** | 20 | NI | 10 | 23 | **436** |
| **HABAT** | 11 | NI | 14 | NI | 18 | 18 | 18 | 25 | NI | 25 | 14 | 23 | 17 | 16 | NI | 20 | NI | 23 | 39 | 16 | 20 | NI | NI | 13 | 22 | NI | NI | 24 | **387** |
| **HABME** | 10 | NI | **22** | NI | 20 | 19 | **26** | 27 | NI | 28 | 12 | 23 | 16 | 15 | NI | 20 | NI | 25 | 33 | 18 | 20 | NI | NI | NI | 26 | NI | NI | 26 | **404** |
| **HABW** | NI | 10 | 15 | NI | 16 | NI | 18 | 25 | NI | 18 | 12 | 23 | 14 | 14 | NI | 18 | NI | NI | 28 | 18 | 16 | **17** | NI | 12 | 26 | NI | NI | 14 | **334** |
| **Max Inhibition** | **14** | **18** | **22** | **17** | **24** | **23** | **26** | **30** | **10** | **28** | **17** | **26** | **20** | **21** | **20** | **21** | **NI** | **29** | **40** | **25** | **24** | **17** | **NI** | **14** | **28** | **NI** | **11** | **29** | |

Note: Inhibition in millimeter

***Proteus sp.***

The Proteus species also showed the susceptibility against all the extracts but any particular extract not shown the dominance in inhibition as it was there in earlier methanolic extract of *E. coli*. The variations in the zone of inhibition recorded when tested with combinations of antibiotics and extracts alone where, maximum zone of 44 ±12 mm was recorded in Bark extract and 42 ±12 mm was in leaves extract of *A. polyacantha.*

Among the bark extracts, CABME has shown the sum of maximum zone of inhibition calculated from all the groups of antibiotics followed by HABCL. The overall decreasing inhibition with extracts was highlighted as follows:
CABME>HABCL>HABPE>HABME>CABAT>CABPE>CABCL>CABW>HABAT>HABOPE>HABOCL>HABW> Standard

In the leaves extracts, CALW has shown the maximum sum of inhibition followed by HALCL. The overall decreasing inhibition with extracts was highlighted as follows:
CALW>HALCL>HALME>CALAT>HALPE>CALME>CALPE>CALCL>HALAT>HALOCL>HALW>HALOPE>Standard

In bark extracts, the number of times highest zone of inhibition recorded in CABW (31 times) followed by CABAT (23times). In leaves extract also, CALW scored (33 times) highest followed by two extracts HALCL and HALME (18 times).

## DISCUSSION

Developing resistance against antibiotics in bacterial species was attempted to control by synergistic action of the plant *A. polyacantha.* The extracts of *A. polyacantha* bark and leaves increased antibiotic susceptibility *in vitro* against *E. coli* and *Proteus* sp. Among them, hot and cold methanolic extract of bark showed maximum growth inhibiting activity and not only that both of these extracts increased susceptibility towards antibiotics to help in overcoming resistance when used in combination. Likewise, for cold extract group, bark methanolic extract proved to be effective and water extract of leaves proved to be superior in increasing susceptibility against antibiotics. This surely remarked the success of synergistic action against the pathogens. Earlier also, many studies highlighted similar success of plant extracts where plant based product's antimicrobial properties recorded for great implication in therapeutic treatments. In last thirty years, a number of studies with several plants have been conducted to prove such efficiency. [12-18]

As per earlier study, *A. polyacantha* contain many secondary metabolites such as saponins, catechic tannins, alkaloids, anthocyans, leuco anthocyanin, coumarins, sterols flavonoids, anthraquinones and terpenes. [19,20]

It has been learnt that with the emergence of multidrug-resistant pathogens, treatment with combinations of antibacterial has become everyday practice.[21]. To develop more valuable chemotherapeutic agents for treating microbial infections, combination therapy becomes a significant strategy as its synergistic interactions can potentially increase efficacy, treat faster, decrease toxicity, avoid the emergence of resistance, and offer broader-spectrum of activity than monotherapy treatments.[22]

## CONCLUSION

Study highlighted the use of *A. polyacantha* plant extracts effectively controlled the resistance demonstrated in the *E. coli* and *Proteus* species. The presence of multidrug resistance was also recorded in Enterobacteriaceae which could also be controlled by using the combinational therapy as recorded in our study.

## REFERENCES


1. Miller-Keane, Enterobacteriaceae. (n.d.) : Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition. (2003). from http://medical-dictionary.thefreedictionary.com/Enterobacteriaceae.
2. Partridge SR, Analysis of antibiotic resistance regions in Gram-negative bacteria, FEMS Microbiol. Rev, 35, 2011, 820-55.
3. Stokes HW, Gillings MR, Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens, FEMS Microbiol Rev, 35, 2011, 790-819.
4. Al-Dhaheri AS, Al-Niyadi MS, Al-Dhaheri AD, et al., Resistance patterns of bacterial isolates to antimicrobials from 3 hospitals in the United Arab Emirates, Saudi Med J, 30, 2009, 618-23.
5. Khorasani G, Salehifar E, Eslami G, Profile of microorganisms and antimicrobial resistance at a tertiary care referral burn centre in Iran: emergence of *Citrobacter freundii* as a common microorganism, Burns, 34, 2008, 947-52.
6. Perez F, Van Duin D, Carbapenem-resistant Enterobacteriaceae: a menace to our most vulnerable patients, Cleve Clin J Med, 80, 2013, 225-33.
7. Santos PRV, Oliveira ACX, Tomassini TCB, Controle microbiógico de produtos fitoterápicos, Rev. Farm. Bioquím, 31, 1995, 35-38.
8. Ellof JN, Which extractant should be used for the screening and isolation of antimicrobial components from plants?, J. Ethnopharmacol, 60, 1998, 1-6.

9. Martinez MJ, Vasquez SM, Espinosa-Perez C, Dias M, HerreraSanchez M, Antimicrobial properties of argentatine A isolated from *Parthenium argentatum*, Fitoterapia, 65, 1994, 371-372.
10. Martinez MJ, Betancourt J, Alonso-Gonzalez N, Jauregui A, Screening of some Cuban medicinal plants for antimicrobial activity, J. Ethnopharmacol, 52, 1996, 171-174.
11. Cruz FG, Roque NF, Giesbrecht AM, Davino SC, Antibiotic activity of diterpenes from *Mikania triangularis*, *Fitoterapia*, 67, 1996, 189-190.
12. Almagboul AZ, Bashir AK, Farouk A, Salih AKM, Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity, Fitoterapia, 56, 1985, 331-337.
13. Artizzu N, Bonsignore L, Cottiglia F, Loy G. Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essencial oil, Fitoterapia, 66, 1995,174-175.
14. Ikram M, Inamul H, Screening of medicinal plants for antimicrobial activities, Fitoterapia, 55, 1984, 62-64.
15. Izzo AA, Di Carlo G, Biscardi D, Fusco R, Mascolo N, Borreli F, Capasso F, Fasulo, MP, Autore G, Biological screening of Italian medicinal plants for antibacterial activity, Phytother. Res, 9, 1995, 281-286.
16. Kubo L, Muroi H, Himejima M, Structure-antibacterial activity relationships of anacardic acids, J. Agri. Food Chem., 41, 1993, 1016-1019,.
17. Shapoval EES, Silveira SM, Miranda ML, Alice CB, Henriques AT, Evaluation of some pharmacological activities of *Eugenia uniflora*, J. Ethnopharmacol, 44, 1994, 136-142.
18. Sousa M, Pinheiro C, Matos MEO, Matos FJ, Lacerda MI, Craveiro AA, Constituintes Químicos de Plantas Medicinais Brasileiras, Universidade Federal do Ceará, Fortaleza, 1991, 385-388.
19. Alain KY, Agbangnan DCP, Yèhouénou B et al., Chemical characterization and biological activities of extracts from two plants (*Cissus quadrangularis* and *Acacia polyacantha*) used in veterinary medicine in Benin, Journal of Pharmacognosy and Phytochemistry, 3(6),2015, 91-96.
20. Okpanachi AO, Goji ADT, Ezekiel I, Musa KY, Tanko Y, Mohammed A et al. Effect of Aqueous-Methanolic Stem Bark Extract of *Acacia polyacantha* on Blood Glucose Levels on Normoglycemic Wistar Rats, International Journal of Animal and Veterinary Advances, 4(3), 2012,163-166.
21. Rybak MJ, McGrath BJ, Combination antimicrobial therapy for bacterial infections. Guidelines for the clinician, Drugs, 52,1996, 390–405.
22. Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis, Clin. Infect. Dis., 39, 2004, 797–802.

**Source of support: Nil, Conflict of interest: None Declared**