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Research Article

Evaluation of Antibacterial Effect of Vernonia Anthelmintica Seed Extract and Its Synergistic Effect with Antibiotics on Resistant Bacterial Strains

*Gauthami Ravula, Sudhakara Reddy Goluguri, Karthik Konanki

**NRI college of pharmacy, Pothavarapadu, Agiripalli, Vijayawada, Krishna district, Andhra Pradesh, India*

ABSTRACT

Development ofdrug resistance by various microbes is an emerging problem now-a-days and the use of several antibiotics has been ineffective in solving the widespread problem.The scope of this study was to investigate an alternative approach to combat resistant bacterial strains, which was evaluated by an in-vitro interaction between a plant extract i.e., Methanolic extract of *Vernoniaanthelmintica* seeds and known antibiotics such as streptomycin and ampicillin. Three different bacterial strainsie.,E.coli, P. aeruginosa, S. aureus were used for evaluating the antimicrobial activity of the methanolic extract of *Vernoniaanthelmintica* seeds, using disc diffusion method after evaluating its phytochemical profile. Standard antibiotics like streptomicin and ampicillin were used to determine the synergistic effect. The bacterial strainsselected were tested for their resistance towards antibiotics i.e., ampicillin and streptomycin. The results of the study were found to be additive and the extract has shown synergistic effect which was greater on the resistant bacterial strains.

Key words: Synergism, bacterial resistance, phytochemical profile, disc diffusion, in-vitro interaction, susceptible

INTRODUCTION

Recent research is focused on natural plant product as an alternative to the existing drugs for disease remedy in developing countries[1]. Infectious disease still remains an important cause of morbidity and mortality in man[2]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by bacteria has increased and has now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents[3].There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases, appearance of undesirable side effects of certain antibiotics as well as the increasing development of resistance and multi drug resistance in current clinical use[4]. The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections and prevention of the emergence of resistant microorganisms. Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported. Ample evidence of the use of individual plant extracts as antibacterial agents are available, but the works on the combined use of extracts is limited.

MDR (Multi Drug Resistance) due to the expression of efflux pumps is an increasing clinical problem, rendering many antibiotics redundant. Novel antibiotics with new modes of action are urgently required to suppress the rise of MDR bacteria. An alternative approach would be to identify molecules that can interfere with the process of efflux. Currently there are no EPI/ antimicrobial drug combinations in the market, although research into identifying potential EPIs is ongoing both in academic institutions as well as the pharmaceutical industry. In this study *Vernoniaanthelmintica* seeds were used to investigate the combined effect of its methanolic extract with antibiotics on resistant bacterial strains.

MATERIALS AND METHODS

1.1. *Collection of plant material:* The seeds of *Vernoniaantihelmintica* were collected from Viruvanagar, Tamilnadu, India and authenticated by Dr. V.Chelladurai, Former Research Officer, Botany, Central Council for Research in Ayurveda and Siddha, Government of India, Palayumkottai.

1.2. *Preparation of methanolic extract:* The dried seeds were powdered and the powder was subjected to soxhlet extraction with solvent methanol (70%) for 6hrs. The residue was concentrated, dried,desiccated and refrigerated until used.

1.3. *Phytochemical evaluation*[5,6]*:*Tests for alkaloidsincluding Dragendroff's, Wagner's, Mayer's and Hager's Test, test for triterpenoids, tests for coumarins, tannins, saponins, flavones, quinones, flavonones, anthocyanins, anthraquinones, phenols, proteins, carbohydrates, aminoacids and glycosides were performed for evaluating thephytoconstituents of methanolic extract of *Vernoniaanthelmintica* seeds(*MEVA*).

Author for correspondence:Email: gauthamipharma@gmail.com

Table-1 Antibacterial potency and synergistic effect of methanolic extract of *Vernoniaanthelmintica* seeds with standard Streptomycin against three different bacterial species.

Zone of inhibition (in mm)			
Bacterial strain	Methanolic Extract (T)	Streptomycin (S1)	Combination 1 (S1+T=c1)
E.Coli	14.2±0.1749	20.2±0.1562	22.6±0.1860
Pseudomonas	11.6±0.1939	18.6±0.2518	24.2±0.1855
Staphylococcus	10.8±0.2135	19.2±0.2191	25.6±0.2015

1.4.*Screening for antibacterial activity and synergistic effect*

1.4.1.*Materials*

*Microorganisms:*Escherichia coli (MTCC-1652)
Pseudomonas aeruginosa (MTCC-400)
Staphylococcus aureus (MTCC-3160), microbial strains were used.

The strains were maintained on agar slants and stored at 2-8°C temperature

*Standard antibiotics:*Streptomycin (Abbott labs) Ampicillin (AnrosePharma)

*Plant Extract:*Methanolic extract of seeds of *Vernoniaanthelmintica(MEVA)*, (1mg/ml).

1.4.2. *Procedure*

*i) Preparation of standard stock solutions and methanolic extract stock solution:*0.1g of streptomycin & ampicillin powder drugs were used to make standard stock solutions of 100µg/ml using distilled water. Similarly 100mg of test extract(*MEVA*) was accurately weighed & dissolved in 10 ml of DMSO. This solution was used to make the test stock solution(1mg/ml). The organisms used for test i.e., E.coli, S.aereus, P.aeruginosa were sub cultured.

iii) Preparation of standard antibiotic discs and discs with plant extract: Sterile discs of 5mm diameter were made by using Whatmann No.1 grade filter paper and were soaked in standard solutions of streptomycin & ampicillin separately for 20 minutes dried and used. Similarly the sterile discs were soaked in stock solutions of test extract*(MEVA)* separately for 20 minutes.

v) Preparation of discs with combination of standard antibiotics and plant extracts: Equal volumes(1:1) of standard streptomycin solution and test extract*(MEVA)* were mixed together. Likewise combination with standard ampicillin solution was also prepared. The sterile discs were soaked in the above solutions separately for 20 minutes in labelled petri plates which were dried and used.

vi) Screening for the antibacterial activity and synergistic effect by disc diffusion method[7]*:* The sub cultured bacterial strains of E.coli, P.aeruginosa and S.aureus were inoculated onto the solidified agar plates by cotton swabbing. After 20 min, the previously prepared sterile paper discs ofmethanolicextract*(MEVA)*were used to evaluate the antibacterial activity against E.coli, S.aereus, and P.aeruginosa cultures separately. The prepared standard antibiotic discs of streptomycin were placed against the test disc at uniform distance of 1.5". For each test solution duplicates were maintained. These plates were incubated at 35°C for 24hrs. Zone of inhibition was measured for each, using an antibiotic zone reader and compared with that of the reference standard.

Later the extract was screened for synergistic activity with standard antibiotics (streptomycin and ampicillin) by the same procedure as mentioned above but by using standard discs and discs with the combinations, separately for streptomycin and ampicillin. Controls were maintained for medium, organism and paper discs. Synergistic effect was considered when combinations exhibited enlargement of combined inhibition zone by 5mm (GalebAdwan, 2008).

RESULTS AND DISCUSSION

2.1. *Phytochemical screening:* The phytochemical screening showed the presence of Alkaloids, Triterpenoids, Tannins, Saponins, Flavones &Flavanones,Quinones and Phenols in the methanolic extract of *Vernoniaanthelmintica*seeds. Alkaloids, flavonoids, saponins and tannins are known to have antimicrobial activity as well as other physiological activities[8]

2.2. *Antibacterial activity and synergistic effect:* From the results obtainedas in table-1 it was reported that the test extract(*MEVA*) and standard Streptomycin have significant antimicrobial action against all the three bacterial strains i.e., E.coli, Pseudomonas aeroginosa, S.aureus.Regarding the results of the combination c1 (*MEVA*+ standard Streptomycin) the inhibition zone diameters(mm) have been increased significantly on all the three bacterial strains when compared to the zones produced by standard Streptomycin alonewhich was especially greater on P.aeruginosa and S.aureusi.e.,approximately 6mm (>5mm). This increased activity of Streptomycin when combined with the test extract(*MEVA)* might be due to the additive effect produced by the extract. This proves that the seed extract of *Vernoniaanthelmintica* has synergistic activity with Streptomycin on P.aeruoginosa and S.aureus.

The results in table-2 reveal that all the 3 bacterial strains were found to be resistant to standard Ampicillin[9]but, the methanolic extract was effective in controlling the growth of all the three bacterial strains. Interestingly, the zones of inhibition of the combination c2 (*MEVA* + standard Ampicillin) were found to be increased by 6 to 7mm (>5mm) in all the three strains. This could be considered as a factor to report that the methanolic extract of *V.anthelmintica* seeds has shown synergistic activity with Ampicillin on Ampicillin resistant strains of E.coli, P.aeuroginosa, S.aureus which is an encouraging outcome to overcome the problem of bacterial resistance against antibiotics.

Table-2 Antibacterial potency and synergistic effect of methanolic extract of *Vernoniaanthelmintica* seeds with standard Ampicillin against three different bacterial species.

Zone of inhibition (in mm)			
Bacterial strains	Methanolic extract (T=a)	Ampicillin (S2)	Combination2 (S2+T=c2)
E.Coli	14±0.1562	9.8±0.1789	20±0.1356
Pseudomonas	12.2±0.1631	8.2±0.2280	14.2±0.1935
Staphylococcus	11.2±0.1855	7.4±0.1673	13.8±0.1860

Note: All the experiments were done in triplicates and the mean ± S.E.M values were taken

CONCLUSION

Many studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics[10]. Inhibition of efflux pumps significantly decreases the level of intrinsic resistance, reverses acquired resistance and results in decreased frequency of emergence of resistance to efflux pump substrates[11]. Efflux pump inhibitors combined with antibiotics strategy is an effective way to solve the problem caused by resistant bacteria[12]. Our results were found to be encouraging and it was revealed that the combined use of plant extracts and antibiotics could be effective ammunition to combat the emerging drug resistance problem.

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REFERENCES

1. OludaroAnthonia and OmoboyuOlumide, Invitro antibacterial potentials and synergistic effect of southwestern Nigerian plant parts used in folklore remedy for salmonella typhi infection. Nature and science, 2010, 8:9
2. Ghalebadwan and Mohammad khanna, Synergistic effects of plant extracts and antibiotics on staphylococcus aureus strains isolated form clinical specimens. Middle east journal of scientific research; 2008, 3(3):134-139
3. Adwan GM, Invitro activity of certain drugs in combination with plant extracts against staphylococcus aureus infections. Pak j med sci, 2008; 24:4
4. Gislene G, G.F.Nascimento, Juliana Locatelli, Paulo C.Freitas, GiulianaL.Silva, Antibacterail activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilain journal of microbiology, 2000;3:247-256
5. Sofowora, A., Guidelines for research promotion and development in traditional medicine. Nig. J. Pharmacy, 1980, 11: 117-118.
6. Harbone, J.B., Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. 2nd Edn., Chapman Hall, New York, 1973, 278.
7. M.K. Lalitha, Manual on Antimicrobial Susceptibility Testing, Annexure III: Reproduced with permission, from NCCLS publication M100-S12 Twelfth Informational Supplement, 2008, ISBN 1-56238- 454 -6
8. Evans, W.C., Trease and Evans Pharmacognosy. 15th Edn., Division of Reed Elsevier India Pvt. Ltd., New Delhi, India, 2005, ISBN-13: 978-81-312-0087-2.
9. Johnson, T. and C. Case, "Chemical Methods of Control," adapted from *Laboratory Experiments in Microbiology, Brief Edition, 4th ed.* Redwood City, CA: Benjamin/Cummings, 1995.
10. Lin, J., L.O. Michel and O. Zhang, CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrobial Agents and Chemotherapy, 2002, 46: 2124-2131
11. Lomovskaya O, Warren MS, Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. Antimicrob Agents Chemother, 2001, 45:105–16.
12. Lomovskaya.O, K.A. Bostian, Practical applications and feasibility of efflux pump inhibitors in the clinic-A vision for applied use. Biochemical Pharmacology, 2006, 7:910-918