Assessment Of Synergistic Effect Of Plant Extract Of *Catharanthus Roseus* And *Azadirachta Indica* Against Different Pathogenic Microbes

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Abstract-

Society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to microbes. Thus considering the vast potentiality of plant as a source of new therapeutic agents, hence this work was conducted to test the synergistic effect of root, stem, leaf and flower extract of Catharanthus roseus and Azadirachta indica against pathogenic bacteria. Assessment of antibacterial activity was done by using agar well and paper disc diffusion method. Extraction of different plant parts was done by using distilled water and methanol. Synergistic effect was tested to evaluate the possibility of new pharmaceuticals.

Keywords: *Catharanthus roseus, Azadirachta indica, E. coli,* Society and bacteria**.**

1. Introduction-Medicinal plant- based drugs owe the advantage of being simple, effective and exhibit broad spectrum of activity **(Chin et al., 2006)**. Medicinal plant products when compared to their synthetic counterparts minimize the adverse side effects. One approach to treat infectious diseases is the use of plant extracts individually and /or as an alternative approach is the use of combination of plant extracts of different plants/parts or antibiotics with plant extracts. Present study includes *C. roseus* and *A. indica* possessing great medicinal value.

C. roseus (Madagascar periwinkle) is an important medicinal plant, belonging to family Apocynaceae. Pharmacological studies have revealed that *C. roseus* contains more than 70 different type of alkaloids [indole alkaloids] and chemotherapeutic agents that are effective in treating various types of cancers-Lung cancer, Uterine cancer, Melanomas, Hodgkin's and non Hodgkin's lymphomas **[Verpoorte, 1998].** It also possesses known antimicrobial, antibacterial, antifungal, antidiabitic, anticancer and antiviral activities (Fig.1)

A. indica is perhaps the most useful traditional medicinal plant in India. Various parts of the *A. indica* tree have been used as traditional ayurvedic medicine in India from time immemorial. Neem oil, the bark and leaf extract have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter (Fig. 2).

Fig.1 Image of *Catharanhs roseus* leaves and flowers

Fig.2 Image of *Azadirachta indica* leaves and flowers

2. Materials and methods

2.1. Culturing, Isolation and Purification:- Pathogenic microbes were inoculated in petridishes containing suitable medium directly and incubated at suitable temperature for suitable time (for bacteria at 35-37º C for 1-2 days). When colonies appeared on medium in petri dishes, they were transferred to other dishes for purification. Subculturing of respective colonies was done till pure culture was obtained.

2.2. Microbes used: A total of three bacteria *viz. B. fusiformis* [gram positive], *E. coli* [gram negative] and *S. aureus* [gram positive] were selected to assess susceptibility patterns against the plant extracts. Bacterial cultures were maintained in NAM slants at 37º C.

2.3. Collection of plant materials: The fully mature *Catharanthus roseus* and *A. indica* flowers leaves, stem, bark and roots were collected from Heera bagh park, Heera bagh (Agra). Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from plant materials using filter paper before they were allowed to dry in shade.

2.4. Extract preparation-

2.4.1. Aqueous extract- Shade dried plant materials were grinded by using grinder. For individual plant extract 2gm powder of plant part was dissolved in 20 ml distilled water, for combined plant extract, 2 gm (1gm *Catharanthus* parts + 1 gm *Azadirachta* parts (as required) ,for whole plant extract .5 g of each part and for combined plant extract of both the plants .25g was dissolved in 20 ml distilled water and left for 48 hrs. at room temperature and then filtered using muslin cloth. The filtrate was collected in fresh sterilized conical flask and used within 24 hrs.

2.4.2. Solvent extract-For the preparation of individual methanolic extract different plant parts viz. root stem leaves and flower. 2g powder was dissolved in 20 ml methanol. For combined plant extract 2gm (1gm of *Catharanthus* parts + 1 gm *Azadirachta* parts) , for whole plant extract .5 g of each part and for combined plant extract of both the plants .25g of each part was dissolved in 20 ml methanol and after 48 hrs. filtered with muslin cloth. The filtrate was collected in fresh sterilized conical flask and used within 24 hrs.

2.5. Antibacterial activity of *Catharanthus roseus* and *Azadirachta indica* on selected bacterial species.

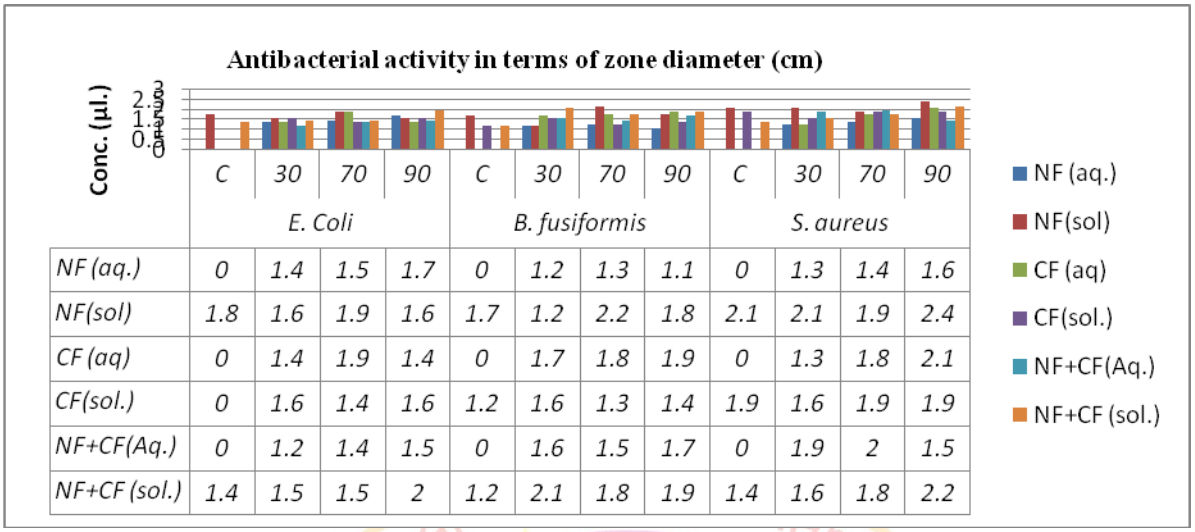
The antimicrobial effect of plant extract viz. *Catharanthus roseus* var. "rosea", and *Azardirachta indica* against the target pathogenic bacteria and fungi was studied in terms of-

2.5.1. Paper disc diffusion method-Disc measuring 06 mm in diameter was pinched from Whatman no. 1 filter paper using a cork borer of fixed diameter. The discs, saturated with different extracts containing varying concentration was placed on NAM medium seeded with the test organism. Disc fed with corresponding solvent alone served as control. The plates were incubated at suitable temperature and observed for zone of inhibition after 1-3 days (**Pelezar et al, 1993; Okigbo et al, 2005**).

2.5.2. Agar well diffusion method- Suitable medium was prepared and poured into sterile Petri dishes. This was allowed to solidify and spread the fungal spores on set petriplates. 5 equi-distant holes per plate were made in the set medium by using a sterile cork-borer of 9 mm diameter. Thereafter, the wells were filled with the extract solution at varying concentrations. This was done in duplicate and plates were incubated at suitable temperature for 1-3 days. The plates were observed for zone of inhibition (cm) around the wells **(Perez et al, 1990)**.

3. Results and conclusion- In this study, two commonly available medicinal plants used by traditional users in India were tested against three bacteria. Different extracts from different plant parts were prepared and assayed

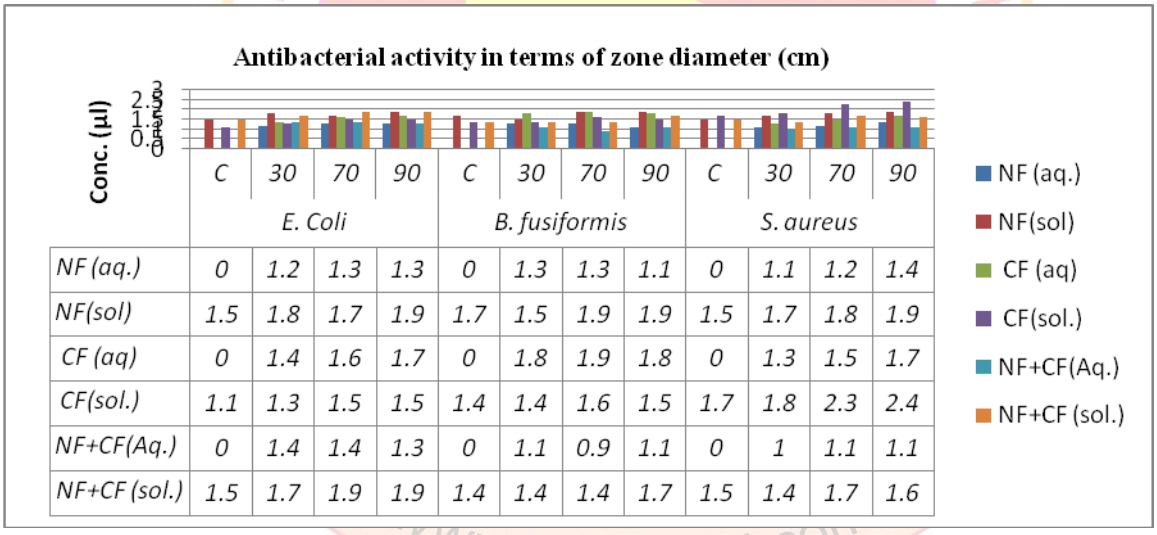
Figure 3.1. Antibacterial activity of flower of *C. roseus* and *A. indica* individually and in combination by Paper disc diffusion method



	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
NF (aq.)	0	1.4	1.5	1.7	0	1.2	1.3	1.1	0	1.3	1.4	1.6
NF(sol)	1.8	1.6	1.9	1.6	1.7	1.2	2.2	1.8	2.1	2.1	1.9	2.4
CF (aq)	0	1.4	1.9	1.4	0	1.7	1.8	1.9	0	1.3	1.8	2.1
CF(sol.)	0	1.6	1.4	1.6	1.2	1.6	1.3	1.4	1.9	1.6	1.9	1.9
NF+CF(Aq.)	0	1.2	1.4	1.5	0	1.6	1.5	1.7	0	1.9	2	1.5
NF+CF (sol.)	1.4	1.5	1.5	2	1.2	2.1	1.8	1.9	1.4	1.6	1.8	2.2

Legends- aq. – Aqueous extract, sol – Methanol extract, C- Control, NF- Neem flower, CF – *C. roseus* flower

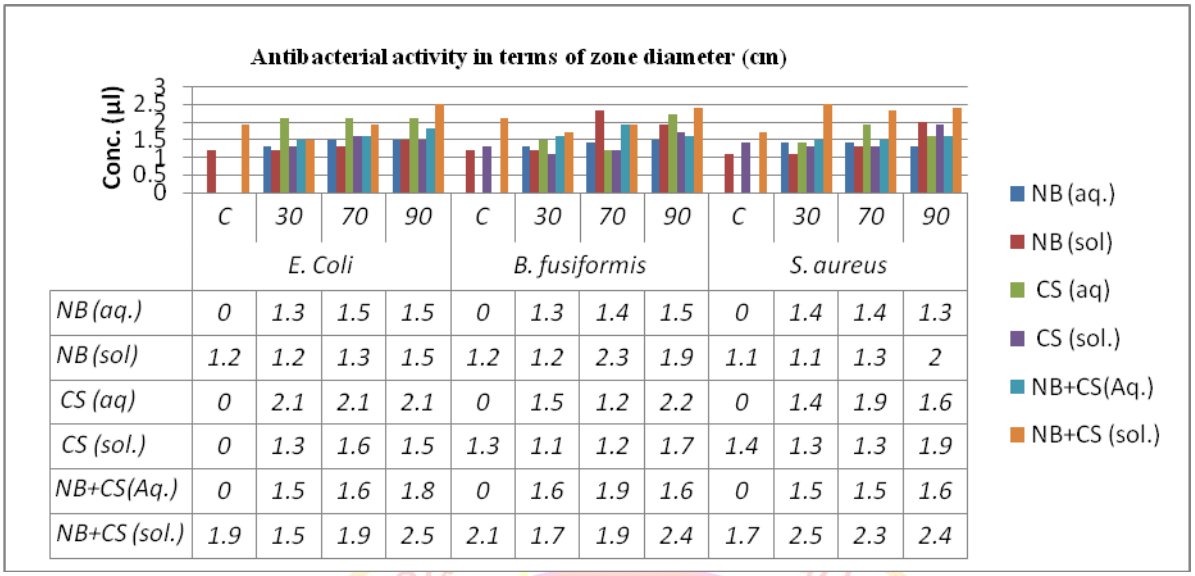
Figure 3.2. Antibacterial activity of flower of *C. roseus* and *A. indica* individually and in combination by Agar well diffusion method



	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
NF (aq.)	0	1.2	1.3	1.3	0	1.3	1.3	1.1	0	1.1	1.2	1.4
NF(sol)	1.5	1.8	1.7	1.9	1.7	1.5	1.9	1.9	1.5	1.7	1.8	1.9
CF (aq)	0	1.4	1.6	1.7	0	1.8	1.9	1.8	0	1.3	1.5	1.7
CF(sol.)	1.1	1.3	1.5	1.5	1.4	1.4	1.6	1.5	1.7	1.8	2.3	2.4
NF+CF(Aq.)	0	1.4	1.4	1.3	0	1.1	0.9	1.1	0	1	1.1	1.1
NF+CF (sol.)	1.5	1.7	1.9	1.9	1.4	1.4	1.4	1.7	1.5	1.4	1.7	1.6

Legends-aq. – Aqueous extract, sol – Methanol extract, C- Control, NF- Neem flower, CF – *C. roseus* flower

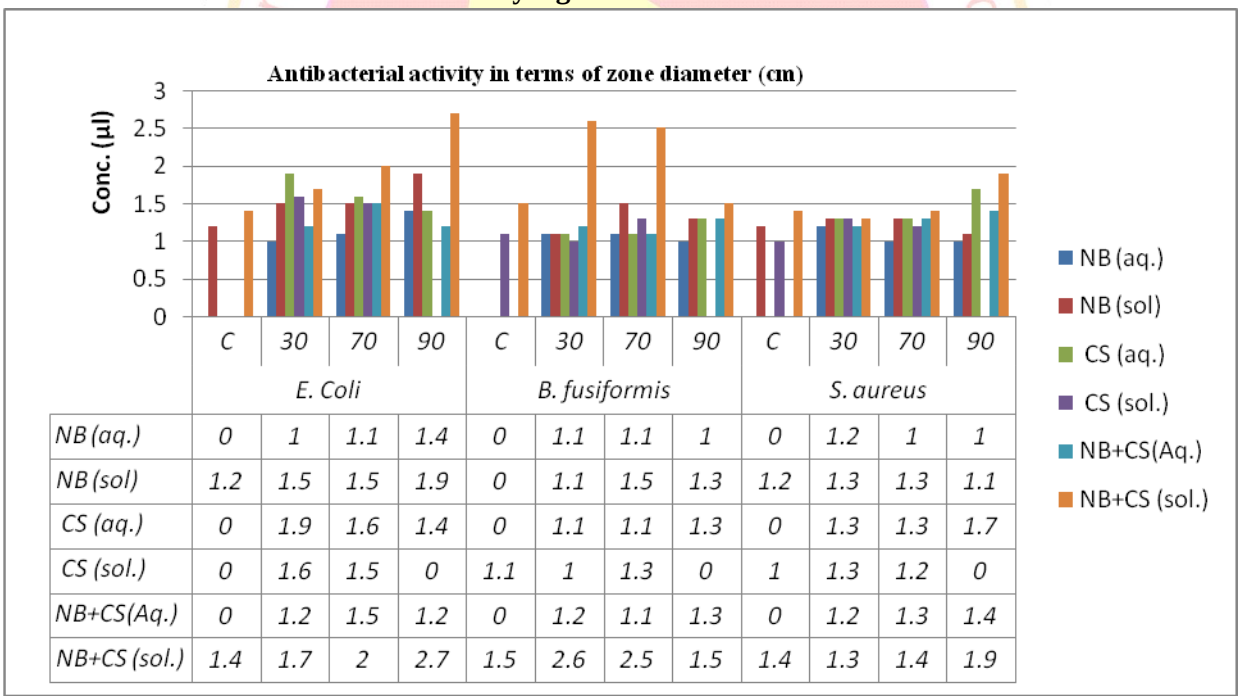
3.3. Antibacterial activity of stem of *C. roseus* and bark of *A. indica* individually and in combination by Paper disc diffusion method



	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
NB (aq.)	0	1.3	1.5	1.5	0	1.3	1.4	1.5	0	1.4	1.4	1.3
NB (sol)	1.2	1.2	1.3	1.5	1.2	1.2	2.3	1.9	1.1	1.1	1.3	2
CS (aq)	0	2.1	2.1	2.1	0	1.5	1.2	2.2	0	1.4	1.9	1.6
CS (sol.)	0	1.3	1.6	1.5	1.3	1.1	1.2	1.7	1.4	1.3	1.3	1.9
NB+CS(Aq.)	0	1.5	1.6	1.8	0	1.6	1.9	1.6	0	1.5	1.5	1.6
NB+CS (sol.)	1.9	1.5	1.9	2.5	2.1	1.7	1.9	2.4	1.7	2.5	2.3	2.4

Legends- aq. – Aqueous extract, sol – Methanol extract, C- control, NB- Neem bark, CS – *C. roseus* stem.

3.4. Antibacterial activity of stem of *C. roseus* and bark of *A. indica* individually and in combination by Agar well diffusion method

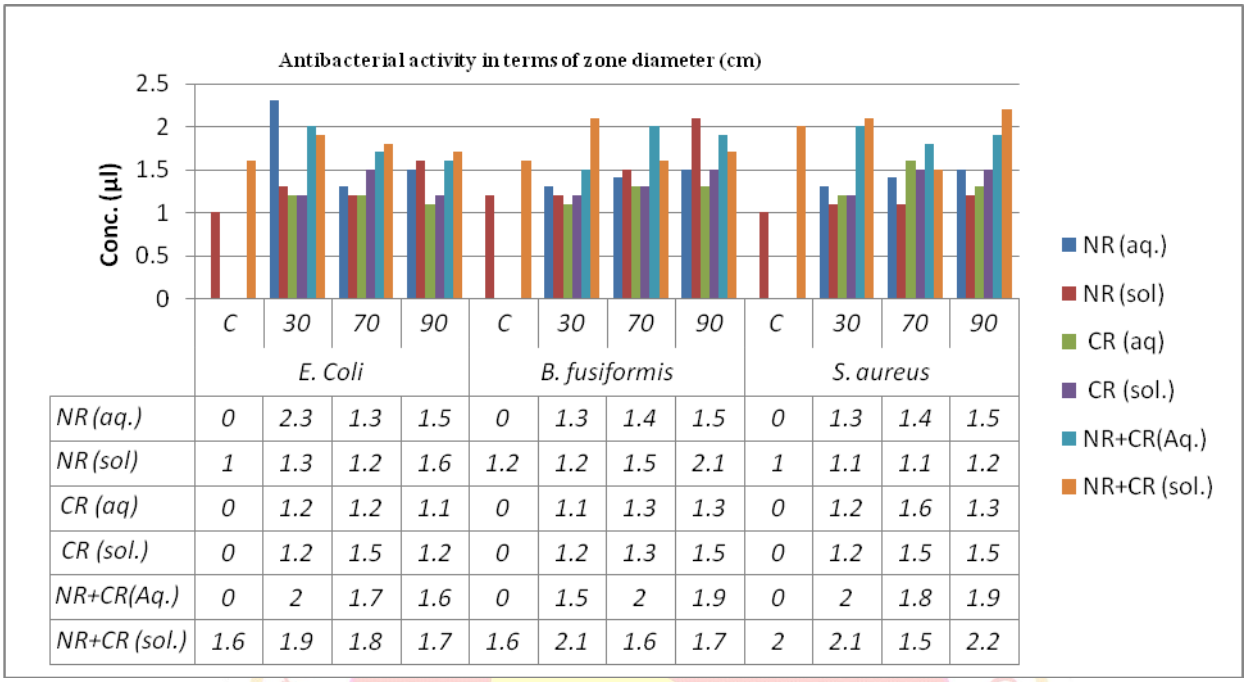


	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
NB (aq.)	0	1	1.1	1.4	0	1.1	1.1	1	0	1.2	1	1
NB (sol)	1.2	1.5	1.5	1.9	0	1.1	1.5	1.3	1.2	1.3	1.3	1.1
CS (aq.)	0	1.9	1.6	1.4	0	1.1	1.1	1.3	0	1.3	1.3	1.7
CS (sol.)	0	1.6	1.5	0	1.1	1	1.3	0	1	1.3	1.2	0
NB+CS(Aq.)	0	1.2	1.5	1.2	0	1.2	1.1	1.3	0	1.2	1.3	1.4
NB+CS (sol.)	1.4	1.7	2	2.7	1.5	2.6	2.5	1.5	1.4	1.3	1.4	1.9

Legends- aq. – Aqueous extract, sol – Methanol extract, C-control, NB- Neem bark, CS – *C. roseus* stem.

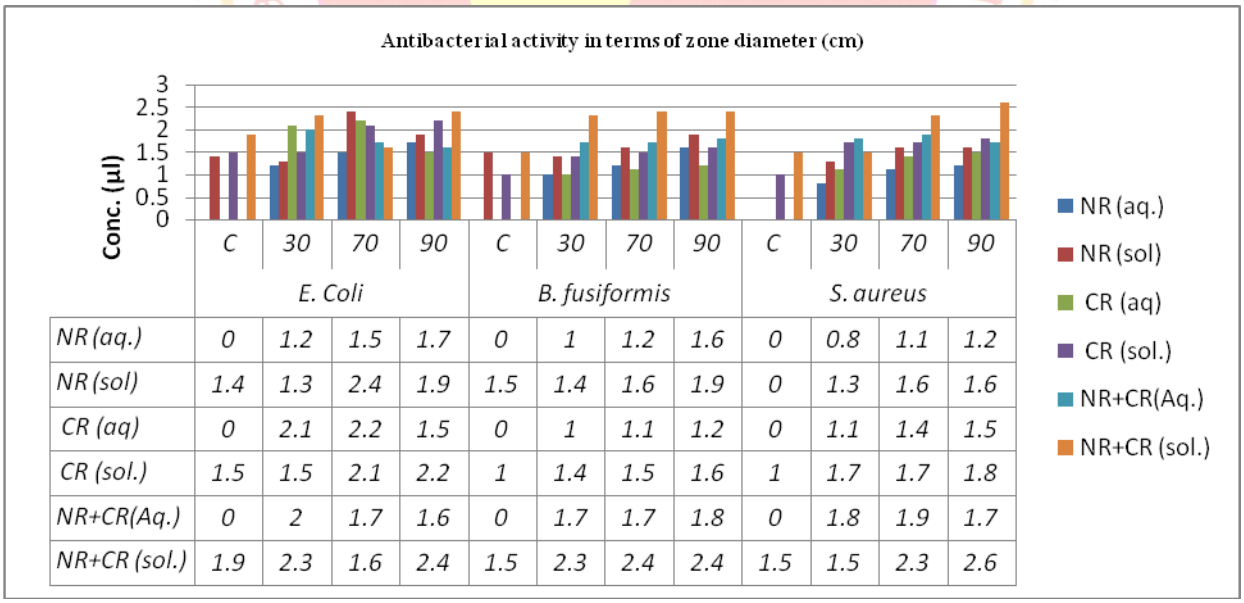
3.5. Antibacterial activity of root of *C. roseus* and *A. indica* individually and in combination by Paper disc diffusion method

Legends- aq. – Aqueous extract, sol – Methanol extract, C-control, NR- Neem root, CR – *C. roseus* root.



	E. Coli				B. fusiformis				S. aureus			
	C	30	70	90	C	30	70	90	C	30	70	90
NR (aq.)	0	2.3	1.3	1.5	0	1.3	1.4	1.5	0	1.3	1.4	1.5
NR (sol)	1	1.3	1.2	1.6	1.2	1.2	1.5	2.1	1	1.1	1.1	1.2
CR (aq)	0	1.2	1.2	1.1	0	1.1	1.3	1.3	0	1.2	1.6	1.3
CR (sol.)	0	1.2	1.5	1.2	0	1.2	1.3	1.5	0	1.2	1.5	1.5
NR+CR(Aq.)	0	2	1.7	1.6	0	1.5	2	1.9	0	2	1.8	1.9
NR+CR (sol.)	1.6	1.9	1.8	1.7	1.6	2.1	1.6	1.7	2	2.1	1.5	2.2

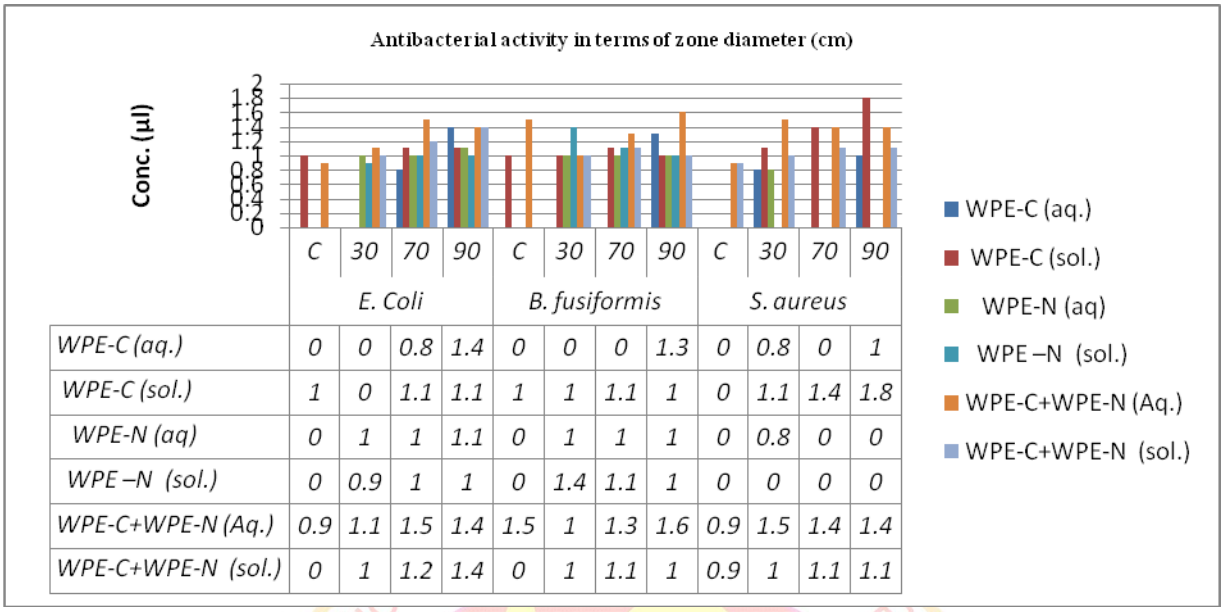
3.6. Antibacterial activity of root of *C. roseus* and *A. indica* individually and in combination by Agar well diffusion method



	E. Coli				B. fusiformis				S. aureus			
	C	30	70	90	C	30	70	90	C	30	70	90
NR (aq.)	0	1.2	1.5	1.7	0	1	1.2	1.6	0	0.8	1.1	1.2
NR (sol)	1.4	1.3	2.4	1.9	1.5	1.4	1.6	1.9	0	1.3	1.6	1.6
CR (aq)	0	2.1	2.2	1.5	0	1	1.1	1.2	0	1.1	1.4	1.5
CR (sol.)	1.5	1.5	2.1	2.2	1	1.4	1.5	1.6	1	1.7	1.7	1.8
NR+CR(Aq.)	0	2	1.7	1.6	0	1.7	1.7	1.8	0	1.8	1.9	1.7
NR+CR (sol.)	1.9	2.3	1.6	2.4	1.5	2.3	2.4	2.4	1.5	1.5	2.3	2.6

Legends- aq. – Aqueous extract, sol – Methanol extract, C-control, NR- Neem root, CR – *C. roseus* root.

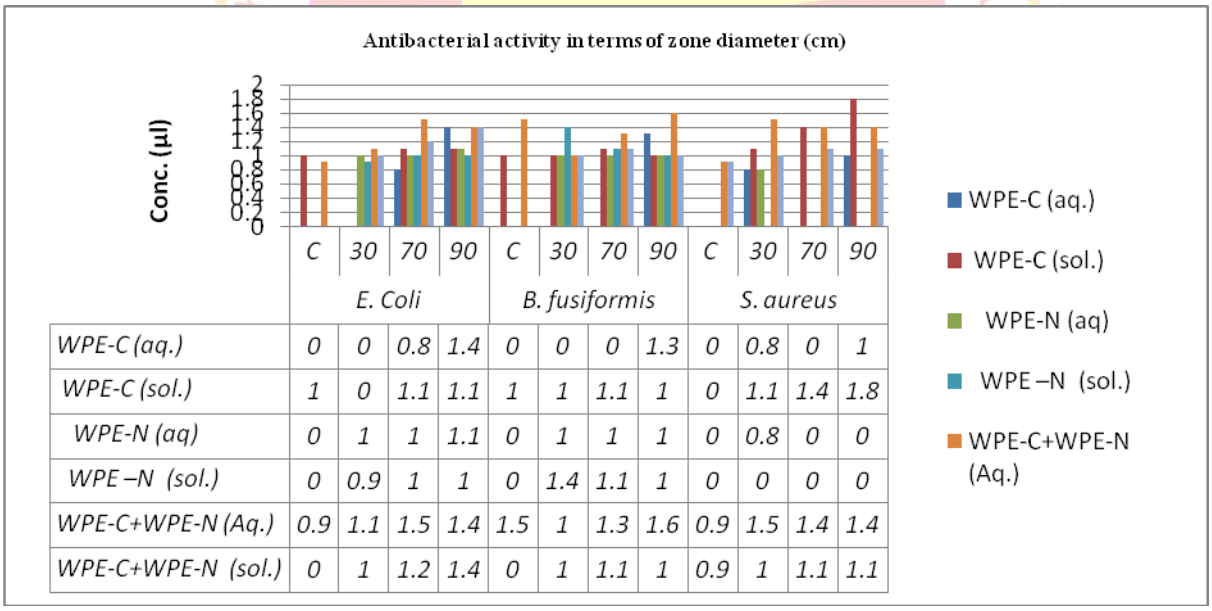
3.7. Antibacterial activity of whole plant extract of *C. roseus* and *A. indica* individually and in combination by Paper disc diffusion method



	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
WPE-C (aq.)	0	0	0.8	1.4	0	0	0	1.3	0	0.8	0	1
WPE-C (sol.)	1	0	1.1	1.1	1	1	1.1	1	0	1.1	1.4	1.8
WPE-N (aq)	0	1	1	1.1	0	1	1	1	0	0.8	0	0
WPE –N (sol.)	0	0.9	1	1	0	1.4	1.1	1	0	0	0	0
WPE-C+WPE-N (Aq.)	0.9	1.1	1.5	1.4	1.5	1	1.3	1.6	0.9	1.5	1.4	1.4
WPE-C+WPE-N (sol.)	0	1	1.2	1.4	0	1	1.1	1	0.9	1	1.1	1.1

Legends- aq. – Aqueous extract, sol – Methanol extract, C- control, WPE-C = Whole plant extract of *C. roseus*WPE-N= Whole plant extract of *A. indica*

3.8. Antibacterial activity of whole plant extract of *C. roseus* and *A. indica* individually and in combination by Agar well diffusion method



	C	30	70	90	C	30	70	90	C	30	70	90
	E. Coli				B. fusiformis				S. aureus			
WPE-C (aq.)	0	0	0.8	1.4	0	0	0	1.3	0	0.8	0	1
WPE-C (sol.)	1	0	1.1	1.1	1	1	1.1	1	0	1.1	1.4	1.8
WPE-N (aq)	0	1	1	1.1	0	1	1	1	0	0.8	0	0
WPE –N (sol.)	0	0.9	1	1	0	1.4	1.1	1	0	0	0	0
WPE-C+WPE-N (Aq.)	0.9	1.1	1.5	1.4	1.5	1	1.3	1.6	0.9	1.5	1.4	1.4
WPE-C+WPE-N (sol.)	0	1	1.2	1.4	0	1	1.1	1	0.9	1	1.1	1.1

Legends- aq. – Aqueous extract, sol – Methanol extract, C-control, WPE-C = Whole plant extract of *C. roseus*, WPE-N= Whole plant extract of *A. indica*

Some plants are known as medicinal because they contain active substances that cause certain reactions, from relenting to the cure of diseases. *C. roseus* and *A. indica* are most important and common medicinal plants. Synergistic effect of different parts of *C.roseus* and *A. indica* was tested against pathogenic bacteria to evaluate the possibility of new pharmaceuticals. The development and spread of drug resistance among different bacteria to currently available antibiotics is a worldwide concern. One strategy employed to overcome these resistance mechanism is the use of combination

therapy. Among all the flower extract against *E.coli* combined methanolic flower extract of both the plants was comparatively more effective using paper disc diffusion method. Individual methanolic extract of flower of *A. indica* was comparatively more effective, in paper disc diffusion method against *B. fusiformis*. Against *S. aureus* individual methanolic flower extract of *A. indica* (paper disc diffusion method) and individual methanolic flower extract of *C. roseus* (agar well diffusion method) was equally effective.

Among stem extract of both plants combined methanolic extract of bark of *A. indica* and stem of *C. roseus* shows positive synergism using agar well method against *E. coli*. Similar positive synergism was reported when the same extract was used against *B. fusiformis* and *S. aureus* in agar well and paper disc diffusion method respectively.

Combined methanolic root extract of both the plants and methanolic root extract of *A. indica* were found to be equally effective against *E. coli* using agar well method. Against *B. fusiformis* and *S. aureus* combination of methanolic extract of root of both plants shows positive synergism, using agar well diffusion method.

Reports of whole plant extract against tested bacteria were not significant as compare to the synergistic effect of two plant parts. The highest inhibition of methanolic extract of whole plant extract of *C. roseus* was reported against *S. aureus* using agar well diffusion method.

Thus, it seems that combination therapy, using multiple plant extracts can be an effective remedy to overcome drug resistance among pathogenic bacteria.

5. References

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