The Journal of Phytopharmacology 2014; 3(2): 102-112
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2014; 3(2): 102-112
March-April

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Identification and characterization of medicinally important plants of Kangra valley with synergistic effects of traditional antibiotics against microbial infections

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Abstract

The use of traditional medicine to treat infection has been practiced since the origin of mankind. In present scenario, the increasing and indiscriminate use of antibiotics has led to the development of microbial resistance to antibiotics. To overcome this, the synergistic effect from the combination of antibiotics with plant extracts against resistant microbes may leads to new ways of treating infectious diseases. This study has been done to evaluate the synergetic effect of common medicinal plants of Kangra valley with traditional antibiotics (Tetracycline, Gentamicin, Streptomycin and Ampicillin). The leaves of different plants i.e *Adhatoda vasica* (Vasaka), *Ficus carica* (Fig), *Calotropis gigantea* (Milkweed), were collected and powdered leaves were extracted successively with methanol, chloroform, petroleum ether and water in soxhlet extractor. Antimicrobial potential of these extract was tested alone and in combination with antibiotics against five bacterial strains (*Bacillus subtilis*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*) and two fungal strains (*Candida albicans* and *Aspergillus brasiliensis*) by well diffusion method. Many combinations showed almost double increased effect like methanolic extract of *Adhatoda vasica* and ampicillin showed synergistic effect against *S. aureus* whereas methanolic extract of Ficus carica showed synergistic effect with gentamycin and tetracyclin against *E.coli* and *S. aureus*. However many combinations do not showed any synergistic effect. Study will help to lay the foundation of searching new antimicrobials and alternatives that are helpful for treating infectious diseases without imparting ill effects and documents the antimicrobial potential of common plants.

Keywords: Antimicrobial, Medicinal plants, Synergistic effects, Infectious diseases, Antibiotics.

Introduction

India, a country of immense biotic wealth, has more than 7000 plant species reportedly used for medicinal purposes most of which are being exploited recklessly for the extraction of drugs. The age old traditional values attached with the various forest types and the varieties of forest products (i.e. medicinal plants) have gained tremendous importance in the present century.[1, 2] Medicinal plants are the most important source of life saving drugs for the majority of the world's population. There are about 45,000 species in India with concentrated Hot Spot is the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Islands. India is the largest producer of

medicinal herbs and is appropriately called the botanical garden of the world.[3]

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. A number of plants have been documented for their medicinal potential, which is in use by the traditional healers, herbal folklorists and in Indian system of medicine namely Siddha, Ayurveda and Unani. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.[4]

Antibiotics are molecules that stop microbes (both bacteria and fungi) from growing or killing them outright. However, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions.[5] Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which there is lesser resistance.[6] As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy.[7]

Synergism is defined as a positive interaction created when two agents are combined and together they exert an inhibitory effect that is greater than the sum of their individual effects. The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death. The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity, reducing the time needed for long-term antimicrobial therapy and prevention of the emergence of resistant microorganisms.[8] Keeping in view the above mentioned fact and observations present study was designed to evaluate the antimicrobial potential and synergy between extracts of some commonly growing plant of Kangra valley of Himachal pradesh with traditional antibiotics and hence to check the medicinal potential of these natural resources to develop new alternative remedies against microbial infections.

Materials and Methods

Collection of sample

The leaves of different plants i.e *Adhatoda vasica* (Vasaka), *Ficus carica* (Fig), *Calotropis gigantea* (Milkweed), were collected from Kangra herbs, Shahpur (H.P.) India.

Preparation of extracts

Plant leaves were taken and dried under shade for 15 days. The dried plant material was crushed into fine powder by help of grinder and stored for required purpose. 40 g of powdered leaves were extracted successively with 200 ml of methanol, chloroform, petroleum ether and aqueous in Soxhlet extractor until the extract was clear. The filtrates were evaporated using vaccum rotary evaporator, and stored at 4°C for further use. Stock solutions of all crude extracts were prepared by diluting the dried extracts with DMSO (Dimethylesulfoxide) to obtain a final concentration of 200 mg/ml.

Collection of microorganism

Microbial strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial strains such as *E. coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 4673), *Streptococcus mutans* (MTCC 890) and *Staphylococcus aureus* (MTCC 3160) were used for antimicrobial assay. All the strains were grown in NB medium and incubated at 37ºC for overnight. Fungal strain *Candida albicans* (MTCC 227) and *Aspergillus brasiliensis* (MTCC 1344) were grown in SDB medium incubated at 25ºC for five day. The entire microorganisms were subcultured in 30 days.

Screening for antimicrobial activities

The antibacterial and antifungal activity was carried out by employing 24 h cultures of *Bacillus subtilis*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis*. The antibacterial and antifungal activity studies were carried out by agar well diffusion method.[9]

All the strains were grown in NB medium and fungal strains were grown in SDB medium. 0.2 ml of bacterial culture broth were inoculated in Mueller-Hinton agar and fungal culture broth were inoculated in Sabouraud Dextrose agar, then poured in petriplates and allowed to solidify. Wells were then bored into the plates of seeded organisms using sterile cork borer of 6 mm in diameter then 50 µl extracts of concentration 200 mg/ml of different solvent were placed on the wells in different plates with a control well with DMSO. All bacterial plates were incubated at 37°C for 24 hrs. and fungal plates at 25°C for five days. The diameter of the minimum zone was measured in mm.

Screening for antimicrobial activity of antibiotics

The antimicrobial activity studies were carried out by well diffusion method. Tetracycline (10 mg/ml), Gentamicin (10 mg/ml), Streptomycin (50 mg/ml) and Ampicillin (50 mg/ml) antibiotics were used. Three wells were bored into the plates of seeded organisms using sterile cork borer of 6 mm in diameter then 50 µl of each antibiotics were placed on the wells in different plates with a control well with DMSO. All bacterial plates were incubated at 37°C for 24 hrs. and fungal plates at 25°C for five days. The zone of inhibition was measured in mm.

Determination of the Synergistic effect Using Well diffusion Method

The combined activity studies were carried out by agar well diffusion method. All the strains were grown in nutrient broth medium and fungal strains were grown in Sabouraud Dextrose broth medium. 0.2 ml of bacterial culture broth were inoculated in Mueller-Hinton agar and fungal culture broth were inoculated in Sabouraud Dextrose agar, then poured in petriplates and allowed to solidify. Wells were then bored into the plates of seeded organisms using sterile cork borer of 6 mm in diameter then 50 µl of each extract and antibiotic were placed on the wells in different plates. All bacterial plates were incubated at 37°C for 24 hrs. and fungal plates at 25°C for five days. The zone of inhibition was measured in mm.

Results

In the present investigation the leaves of different plants i.e *Adhatoda vasica* (Vasaka), *Ficus carica* (Fig) and *Calotropis gigantea* (Milkweed) were collected from Kangra herbs, Shahpur (H.P.). The different types of solvent were used for the extraction of active agents from plant leaves. Solvents such as methanol, petroleum ether, chloroform and aqueous were used for the investigation of the antimicrobial potential of plant leaves.

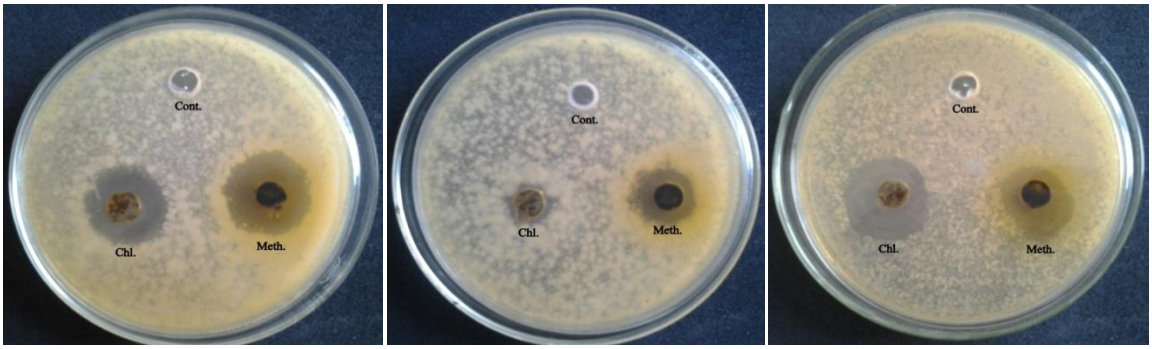
Adhatoda vasica extracts:

Table1.1.A shows the antibacterial activity of different solvent extracts of vasaka (Adhatodavasica). The methanol extracts showed antibacterial activity against all bacterial pathogens, maximum zone of inhibition was showed against *B. subtilis* (17 mm) while petroleum ether, aqueous extracts of vasaka did not show inhibitory effect against tested bacteria. Chloroform extract showed activity against *B. subtilis* and *P. aureuginosa* and maximum zone of inhibition was against *P. aureuginosa* (20 mm) (Figure 1).

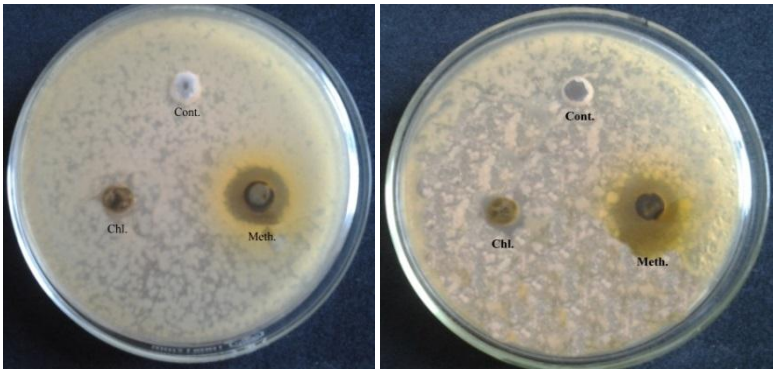
Table 1.1.B showed the antifungal activity of different solvent extracts of vasaka (Adhatoda vasica). Methanol extract also showed antifungal activity against *Aspergillus brasiliensis* and *Candida albicans* whereas chloroform extract showed antifungal activity only against *Candida albicans*. While remaining two extract i.e aqueous and petroleum ether showed no antifungal activity (Figure 2).

Table 1.1.A: Antibacterial activity of various extract of *Adhatoda vasica* against bacterial pathogens

Pathogens	**Sample 1 (S1) (*Adhatoda vasica*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S1-A	Control	S1-B	Control	S1-C	Control	SI-D	Control
B. subtilis	14	-	17	-	-	-	-	-
S. mutans	-	-	10	-	-	-	-	-
P. aureuginosa	20	-	14	-	-	-	-	-
E. coli	-	-	10	-	-	-	-	-
S. aureus	-	-	10	-	-	-	-	-



(Bacillus subtilis) ***(Streptococcus mutant)*** ***(P. aureuginosa)***

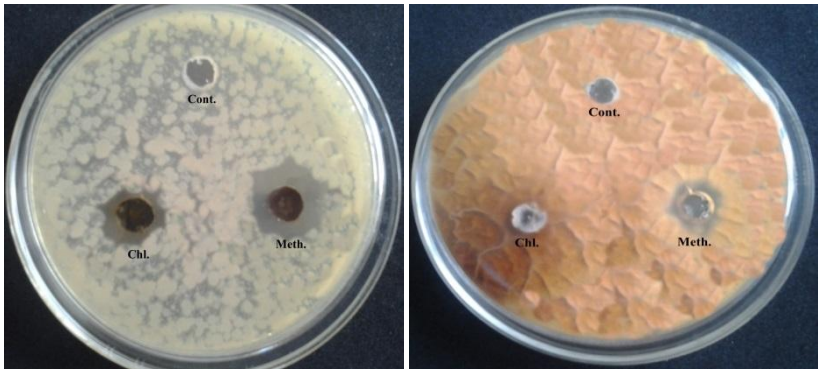


(E. coli) ***(S. aureus)***

Figure 1: Antibacterial activity of various extracts of *Adhatoda vasica* against pathogens

1.1.B: Antifungal activity of various extract of Adhatodavasica against pathogens

Pathogens	**Sample 1 (S1) (*Adhatodavasica*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S1-A	Control	S1-B	Control	S1-C	Control	SI-D	Control
Candida albicans	7.5	-	15	-	-	-	-	-
Aspergillusbrasiliensis	-	-	7	-	-	-	-	-



(Candida albicans) ***(Aspergillus brasiliensis)***

Figure 2: Antifungal activity of various extracts of *Adhatoda vasica* against pathogens

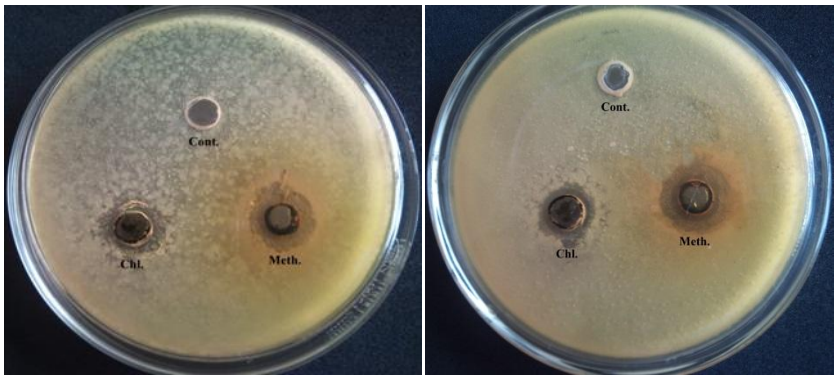
Ficus carica extracts

Table 1.2.A shows the antibacterial activity of different solvent extracts of Fig (*Ficus carica*). The Chloroform extract showed antibacterial activity against all the five bacterial cultures. Chloroform showed antibacterial activity against only two bacterial cultures i.e *B. subtilis*, *Streptococcus mutant*. Other two extracts showed no antibacterial activity against the tested organism (Figure 3).

Table 1.2.B shows the antifungal activity of different solvent extracts of Fig (*Ficus carica*). The chloroform, aqueous and petroleum extracts showed activity only against *Candida albicans* and methanol extract showed activity against *Aspergillus brasiliensis* (12 mm) only. Remaining two extracts i.e aqueous and petroleum ether was not shown antifungal activity against tested pathogens (Figure 4).

Table 1.2.A: Antibacterial activity of various extract of *Ficus carica* against bacterial pathogens

Pathogens	**Sample 2 (S2) (*Ficus carica*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S2-A	Control	S2-B	Control	S2-C	Control	S2-D	Control
B. subtilis	7	-	-	-	-	-	-	-
S. mutans	6	-	-	-	-	-	-	-
P. aureuginosa	-	-	-	-	-	-	-	-
E. coli	8	-	9	-	-	-	-	-
S. aureus	6	-	11	-	-	-	-	-

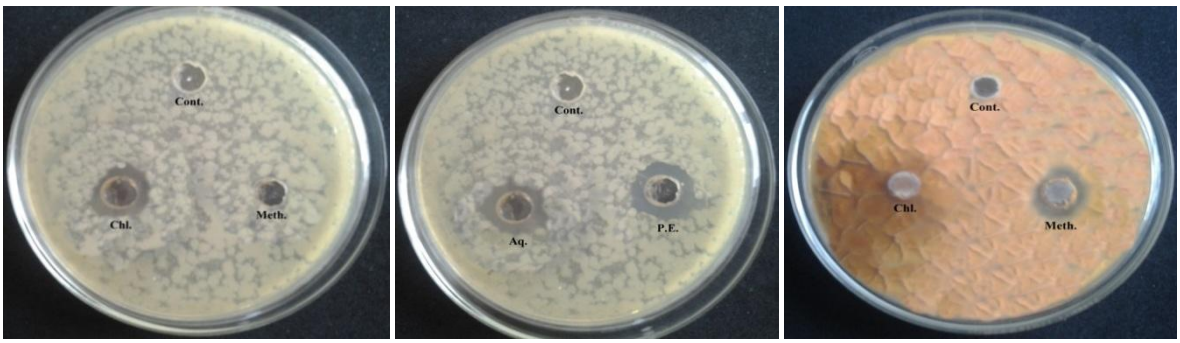


(E. coli) ***(S. aureus)***

Figure 3: Antibacterial activity of various extracts of *Ficus carica* against pathogens

Table 1.2.B: Antifungal activity of various extract of *Ficus carica* against pathogens

Pathogens	**Sample 2 (S2) (*Ficus carica*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S2-A	Control	S2-B	Control	S2-C	Control	S2-D	Control
Candida albicans	6.5	-	-	-	8	-	10	-
Aspergillus brasiliensis	-	-	12	-	-	-	-	-



(Candida albicans) ***(Candida albicans)*** ***(Aspergillus brasiliensis)***

Figure 4: Antifungal activity of various extract of *Ficus carica* against pathogen

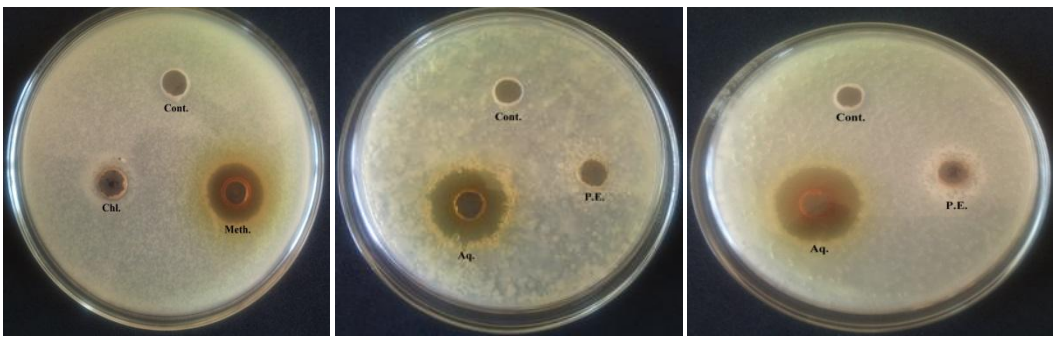
Calotropis gigantea extracts

Table 1.3.A showed the antibacterial activity of different solvent extracts of Milkweed (*Calotropis gigantea*). The aqueous extract showed antibacterial activity against all the four bacterial pathogens except *B. subtilis* and methanol extract showed the antibacterial activity only against *B. subtilis*. Highest zone of inhibition was shown by aqueous extract against *Streptococcus mutant* (18 mm). Chloroform and petroleum ether extracts were not showed antibacterial activity against the organisms tested (Figure 5).

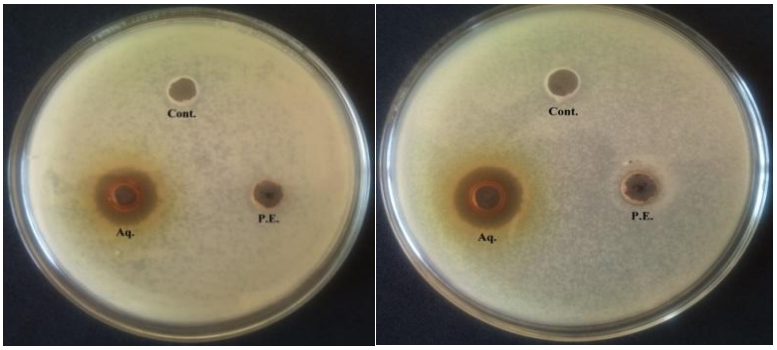
Table 1.3.B shows the antifungal activity of different solvent extracts of Milkweed (*Calotropis gigantea*). The methanol extracts showed activity against both *Aspergillus brasiliensis* (13.5 mm) and *Candida albicans* (17 mm). Aqueous extracts showed activity against only Candida albicans. The chloroform and petroleum ether extracts were not showed antibacterial activity against the pathogens tested (Figure 6).

Table1.3.A: Antibacterial activity of various extract of Calotropis gigantea against bacterial pathogens

Pathogens	**Sample 3 (S3) (*Calotropis gigantea*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S3-A	Control	S3-B	Control	S3-C	Control	S3-D	Control
B. subtilis	-	-	9	-	-	-	-	-
S. mutans	-	-	-	-	18	-	-	-
P. aureuginosa	-	-	-	-	16.5	-	-	-
E. coli	-	-	-	-	12	-	-	-
S. aureus	-	-	-	-	10	-	-	-



(B. subtilis) ***(Streptococcus mutant)*** ***(P. aureuginosa)***

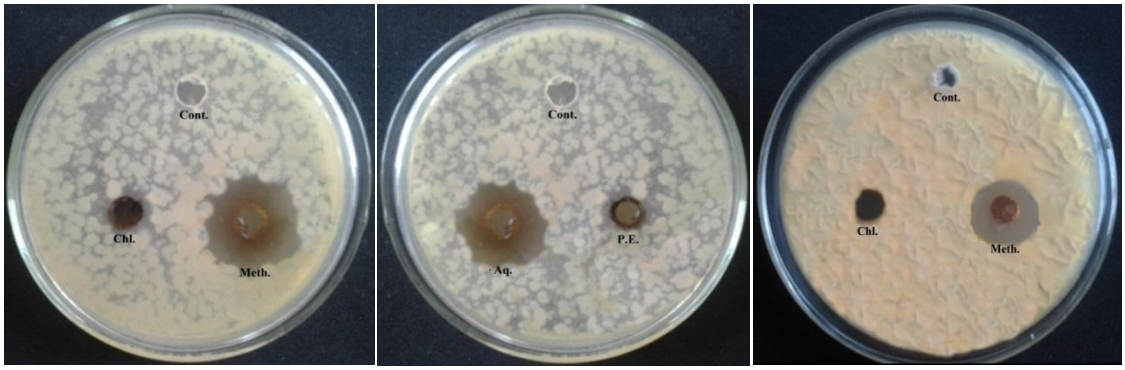


(E. coli) ***(S. aureus)***

Figure 5: Antibacterial activity of various extracts of *Calotropis gigantea* against pathogens

Table 1.3.B: Antifungal activity of various extract of *Calotropis gigantean* against pathogens

Pathogens	**Sample 3 (S3) (*Calotropis gigantea*) Zone of inhibition in mm**							
	Chloroform (A)		**Methanol (B)**		**Aqueous (C)**		**Petroleum ether (D)**	
	S3-A	Control	S3-B	Control	S3-C	Control	S3-D	Control
Candida albicans	-	-	17	-	15.5	-	-	-
Aspergillus brasiliensis	-	-	13.5	-	-	-	-	-



(Candida Albicans) ***(Candida Albicans)*** ***(Aspergillus brasiliensis)***

Figure 6 Antifungal activity of various extract of *Calotropis gigantea* against pathogen

Table1.4 Four different types of antibiotics used in this study. All antibiotics show zone of inhibition.Tetracycline showed maximum zone of inhibition against *P. aureuginosa*, *S. aureus* and *Aspergillus brasiliensis*, gentamicin showed maximum zone of inhibition against *streptococcus*, streptomycin showed maximum zone of inhibition against *E.coli* and ampicillin showed maximum zone of inhibition against *streptococcus* and *Aspergillus brasiliensis*.

Table 1.4: Antimicrobial activity of various antibiotics against pathogens

Pathogens	**(Antibiotics) Zone of inhibition in mm**			
	Tetracycline	**Gentamicin**	**Streptomycin**	**Ampicillin**
B. subtilis	17	22	18	16
S. mutans	17	30	22	25
P. aureuginosa	27	29	25	20
E. coli	26	29	34	15
S. aureus	27	29	29	14
Candida albicans	25	29	30	22
Aspergillus brasiliensis	27	22	20	25

Synergistic effect of different plant extract with antibiotics

Table 1.5 A, B and C shows the synergistic effect of plant extracts and different antibiotics. The plant extracts which showed zone of inhibition were combined with the different antibiotics. Synergistic effect was not showed by all the combinations. In case of *Adhatoda vasica*, methanolic extract was showed the synergistic effect with ampicillin against *S. aureus*. In case of *Ficus carica*, methanolic extract was showed the synergistic effect with gentamicin against *E.coli* and also showed the synergistic effect with ampicillin against *S. aureus* (Figure 7).

Table1.5.A: Synergistic effect of Adhatoda vasica with antibiotics

Pathogens	***Adhatoda vasica* (Plant extract + Antibiotics) Zone of inhibition in mm**															
	Chloroform (A)				**Methanol (B)**				**Aqueous (C)**				**Petroleum ether (E)**			
	T	G	S	A	T	G	S	A	T	G	S	A	T	G	S	A
B. subtilis	16	22	18	16	16	20	18	16	-	-	-	-	-	-	-	-
S. mutans	-	-	-	-	17	30	20	24	-	-	-	-	-	-	-	-
P. aureuginoa	27	29	25	18	25	29	25	20	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	24	29	34	15	-	-	-	-	-	-	-	-
S. aureus	-	-	-	-	27	29	29	18	-	-	-	-	-	-	-	-
Candida albicans	24	29	30	22	25	28	30	22	-	-	-	-	-	-	-	-
Aspergillusbrasiliensis	-	-	-	-	27	21	18	25	-	-	-	-	-	-	-	-

In Table 1.5 A: T- Tetracycline; G-Gentamycin; S-Streptomycin; A-Ampicillin

Table1.5.B: Synergistic effect of Ficus carica extract with antibiotics

Pathogens	***Ficuscarica* (Plant extract + Antibiotics) Zone of inhibition in mm**															
	Chloroform (A)				**Methanol (B)**				**Aqueous (C)**				**Petroleum ether (E)**			
	T	G	S	A	T	G	S	A	T	G	S	A	T	G	S	A
B. subtilis	17	22	18	14	-	-	-	-	-	-	-	-	-	-	-	-
S. mutans	16	32	22	25	-	-	-	-	-	-	-	-	-	-	-	-
P. aureuginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	26	29	29	13	25	32	28	15	-	-	-	-	-	-	-	-
S. aureus	27	28	29	14	27	29	27	18	-	-	-	-	-	-	-	-
Candida albicans	24	29	30	21	-	-	-	-	25	28	20	22	24	29	29	22
Aspergillusbrasiliensis	-	-	-	-	26	22	20	25	-	-	-	-	-	-	-	-

In Table 1.5.B: T- Tetracycline; G-Gentamycin; S-Streptomycin; A-Ampicillin

Table1.5.C: Synergistic effect of Calotropis gigantea extract with antibiotics

Pathogens	***Calotropisgigantea* (Plant extract + Antibiotics) Zone of inhibition in mm**															
	Chloroform (A)				**Methanol (B)**				**Aqueous (C)**				**Petroleum ether (E)**			
	T	G	S	A	T	G	S	A	T	G	S	A	T	G	S	A
B. subtilis	-	-	-	-	16	22	18	15	-	-	-	-	-	-	-	-
S. mutans	-	-	-	-	-	-	-	-	17	32	21	25				
P. aureuginosa	-	-	-	-	-	-	-	-	27	28	25	20	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	26	29	33	14	-	-	-	-
S. aureus	-	-	-	-	-	-	-	-	26	29	28	14	-	-	-	-
Candida albicans	-	-	-	-	-	-	-	-	25	28	31	21	-	-	-	-
Aspergillusbrasiliensis	-	-	-	-	-	-	-	-	27	21	20	22	-	-	-	-

In Table 1.5 C: T- Tetracycline; G-Gentamycin; S-Streptomycin; A-Ampicillin

Adhatodavasica (*S.aureus)* (Methanol+Ampicillin)

Ficuscarica (E.coli) (Methanol+Gentamicin)

Ficuscarica(*S.aureus)* (Methanol+Ampicillin)

Figure 7: Synergistic effect of different plant extract with antibiotics

Discussion

The indiscriminate use of antibiotics has made many microorganisms develop resistance to them. This has created immense clinical problems in the treatment of infectious diseases. Therefore, there is a need to develop alternative antimicrobial agents for the treatment of infectious diseases. Medicinal and aromatic plants have played an important role in the socio cultural, spiritual and healthcare needs of the rural and tribal people and their live stocks in the emerging and developing countries. Antibiotics, in combination of plant extracts can perform better than the addition value. Some of the advantages of synergistic interactions of plants and antibiotics are increased efficiency, reduction of undesirable effects, increase in stability or bioavailability of the free agents and obtaining an adequate therapeutic effect with relatively small doses when compared with synthetic medication.[10] Synergistic effects resulting from the combination of antibiotics with various plant extracts has been studied and experimented by a number of scientists. Sometimes the use of single antibiotic does not produce the desired or the effective inhibitory effects and to overcome this, combination of drugs often exercise their synergistic effect which surpasses their individual performance.[11, 12] In the present investigation, Synergistic effect of plant extracts of *Adhatoda vasica*, *Ficus carica* and *Calotropis gigantea* against five bacterial strains *E. coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 4673), *Staphylococcus aureus* (MTCC 3160) and two fungal strains *Candida albicans* (MTCC 227), *Aspergillus brasiliensis* (MTCC 1344) has undertaken. The combination of methanolic extract of *Adhatoda vasica* (10 mm) with ampicillin (14 mm) showed the synergistic effect against *S. aureus* (18 mm). This enhanced effect may results from the combined action of ampicillin with major alkaloids vasicinone and vasicinol present majoraly in methanollic extract which are having the inhibitory activity against *S. aureus*. Similar was the case with the combination of methanolic extracts of *Ficus carica* (9 mm) with gentamycin (29 mm) with which exhibited synergistic effect against *E. coli* (32 mm) that may be attribute to the presence of coumarins[13] in the mehanollic extracts of *Ficus carica*.[14] Ampicillin alone could cause the inhibition of *S. aureus* which was 14 mm whereas when it was combined with methanolic extract of *Ficus carica* (11 mm), it showed synergy (18 mm) that can be attribute to the fact that it is rich in triterpenoids like bauerenol, lupeol acetate, methyl maslinate, and oleanolic acid.[14] Many workers have reported such synergestic effects in other plants also.[15-17] The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death. Thus it is concluded that to control a particular disease in vitro experiments should be carried out with various antibiotics and their combination as well as antibiotics and plant extracts, so that a right combination may be administered to the patient for early and safe recovery from a specific ailment. All the combinations do not produce synergistic effect and therefore a number of combinations are required to be tested. For determining the synergistic effect, we have only combined those plant extracts with antibiotics which showed antimicrobial activity against different microbial pathogens.

Conclusion

Natural products are in use for the treatment of infectious diseases since times immemorial and plants have been an integral part of traditional medicinal system all over the world. Recent years have witnessed a renewed interest in homemade remedies as an impressive number of modern drugs developed from plants. This study emphasizes synergistic effect of different common plant extract against human pathogenic bacteria. This study suggested that herbs with unique chemical compounds that can either inhibit the growth of pathogens or kill them can be considered as potential candidates for developing new antimicrobial drugs. It is interesting that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species and collection site, and also there are differences in the resistance imparted by microorganisms to some plants, due to the cell wall structure, species and subspecies. According to our results, Methanolic extract of *Ficus carica* showed the synergistic effect with gentamicin against *E. coli* and ampicillin against *S. aureus* respectively. Methanolic extract of *Adhatoda vasica* also showed synergistic effect against *S. aureus*. This study will help to lay the foundation of searching new antimicrobials and alternatives that are helpful for treating infectious diseases without imparting ill effects using common plants.

Acknowledgement

We are very thankful to Hon'ble Vice chancellor and management Arni University for providing necessary

facility. The cooperation of Biotechnology Department is duly acknowledged.

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