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Research Article

EFFECT OF SOME MEDICINAL PLANTS ON OPPORTUNISTIC BACTERIAL AND FUNGAL PATHOGENS ASSOCIATED WITH HIV

B BHARATHI*[1], K SUNDARI[2], G SWAMIDOSS DANIEL[1]

[1] Department of Microbiology, PRIST University, Thanjavur - 614 904, India.
[2] Department of Biochemistry, PRIST University, Thanjavur - 614 904, India
E-mail: bharathikarnan@gmail.com

ABSTRACT

The present study aims to investigate the effect of some easily available medicinal plants on HIV related opportunistic bacterial and fungal pathogens. Opportunistic infections are most common in immunocompromised patients which are the leading cause of death in HIV-infected patients. Antibacterial activity of ethanol and DMSO extracts of 6 medicinal plants was investigated by well-diffusion method against pathogens associated with HIV. The plant extracts showed antibacterial and antifungal activity against the tested organisms. Ethanolic leaf extract of methanolic extract showed highest inhibitory activity when compared with all treatments. Phytochemical screening of six medicinal plants showed positive results for mostly all phytochemical constituents namely tannins, saponins, flavonoids, carbohydrates, alkaloids, anthraquinones. Hence this study provided a good medicinal plant based treatment strategy and will create social awareness among the HIV patients. When compared with antibiotic therapy, the herbal extracts(based medicines) with different concentrations produce better inhibitory action for bacterial and fugal infections.

Key words: Medicinal plants, HIV, Opportunistic infections, Well diffusion method, Phytochemical screening.

INTRODUCTION

In developing countries, microorganisms are frequently a cause of prevailing diseases, presenting a serious public health issue in a significant segment of the population as uncovered by either private or official health care systems. People with advanced HIV infections are vulnerable to infections and malignancies are called "opportunistic infections" [1]. Many bacterial pathogens, including *Mycobacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Campylobacter*, *Listeria* and *Legionella*, *Haemophilus*, *Pseudomonas*, *Rhodococcus* and *Salmonella* are most common in persons infected with HIV. Opportunistic fungal infections are commonly encountered in the AIDS population [2]. The majority of HIV patients 77-100% are infected with *C.albicans*[3].

Baceterial and fungal infections are a major cause of morbidity and mortality in HIV patients[4]. Plants continue to be major resources for therapeutic compounds. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development [5]. Antibiotics can literally save lives and are effective in treating illnesses,however, they have the potential to cause unwanted side effects. Fungal infection of the mouth, digestive tract and vagina can also occur with antibiotics because they destroy the protective good bacteria in the body as well as the bad ones responsible for the infection being treated. Medicinal plant products when compared to their synthetic counterparts minimize the adverse side effects [6]. Hence in this study, six medicinal plants namely *Acalypha indica, Acorus calamus, Adathoda vasica, Azadirachta indica, Calotropis procera* and *Vitex negundo* were selected based on the availability to test the antibacterial and antifungal activity by well diffusion method.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [7] .

MATERIALS AND METHODS

Collection of Samples

Biosamples namely blood, urine, sputum, stool, pus and wound, nail and head scrapings were collected from the suspected symptomatic HIV positive patients. All the strains were confirmed by cultural and biochemical characteristics. CD_4count was done for the HIV patients.

Collection of Plant material

Fresh leaves of plants belong to 5 plants namely *Acalypha indica, Acorus calamus, Adhatoda vasica, Azadirachta indica , Vitex negundo* L and root materials of 1 plant *Calotropis procera* were tested in this study. All plants were collected from Herbal gardens of PRIST University, Thanjavur, the identity of the plants confirmed by a botanist. Plant materials namely the leaf and root parts of all medicinal plants were collected, cleaned and shade dried and powdered.

Plant extraction and Phytochemical Screening

The crude plant extracts were obtained by using Soxhlet apparatus. 2 types of solvents has been used namely ethanol and DMSO. For the collection of crude extract classical method was performed by using rotatory shaker. Qualitative Chemical tests were carried out on the methanolic extract of the plant using standard procedures to identify the phytoconstituents as described by Trease and Evans(1978) [8] and Sofowara (1993) [9].

Isolation and identification of pathogens

Bacterial and fungal strains tested in this study were isolated from clinical cases of suspected symptomatic HIV patients. Six bacterial pathogens namely *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus* and one fungal spp namely *Candida albicans* were confirmed by cultural and biochemical characteristics.

Antimicrobial activity

The antimicrobial assay of ethanol DMSO extracts was performed by agar well diffusion method[10]. 3 types of concentrations were used for bacteria namely 10mg/10ml, 10mg/1ml and 50mg/1ml of plant extract dissolved in Dimethylsulfoxide. In 10mg/10ml , 3 types of concentrations used such as [for 10-100mg] 10mg/10µl, 50mg/50 µl, 100mg/100 µl. In 10mg/1ml 3 types of concentrations used were [for 100mg/1000mg], 250mg/25 µl, 500mg/50 µl and 1000mg/100 µl. In 50mg/1ml, three types of concentrations used namely [for 1000-2000mg], 1250mg/25 µl, 1500mg/30 µl, 2000mg/40 µl. For fungi, 10-100mg(10mg/10ml) was used. For each concentration, extract amount viz., 10µl, 50 µl and 100µl used. For each concentration, controls were included that comprised pure solvents instead of the extracts. The plates were then incubated at 37^0 for 24 hours. The diameter of inhibition zones was measured.

RESULTS

Table 1 showed the isolated pathogens with CD4 counts. Five major bacterial pathogens were known to be isolated from the samples. Among the bacterial pathogens, *Pseudomonas aeruginosa*(30%) showed highest incidence followed by *Escherichia coli*(24%),

Klebsiella pneumoniae(12%),*Streptococcus pneumoniae*(6%) and *Staphylococcus aureus*(6%). Only one fungal spp., was identified and isolated namely *Candida albicans*(18%) known to be found among the HIV patients during the study period. Table 2 showed the morphology, staining and biochemical characteristics of the isolated bacterial pathogens. Table 3 showed morphological features of isolated *Candida* spp.

EFFECT OF MEDICINAL PLANTS ON ISOLATED PATHOGENS

Effect of ethanolic solvent on bacterial pathogens

Antibacterial activity of ethanol extract of medicinal plant against *P.aeruginosa* represented in Table 4. Highest zone of inhibition(27mm) was observed in 40µl(50mg/1ml) for *Adhatoda vasica*, followed by 24mm was noted in 50µl(10mg/1ml) for *Az.indica*. Antibacterial activity of ethanol extract of medicinal plants against *E.coli* represented in Table 5.

The highest zone of inhibition(26mm) was noted in the concentration of 100µl(10mg/10ml) for *Ac.indica* followed by 18mm was noted in 30µl(50mg/1ml) and 100µl(10mg/1ml) for *A. calamus* and *Az.indica.* Antibacterial activity of ethanolic extract of medicinal plant against *K.pneumoniae* represented in Table 6.

The highest zone of inhibition(21mm) was noted in the concentration of 100µl(50mg/1ml) and 10µl(10mg/10ml) for *A.calamus* and *C.procera*. Antibacterial activity of ethanolic extract of medicinal plants against the isolated *S.pneumoniae* represented in Table 7. Highest antibacterial activity about 18mm was observed in 100µl of (10mg/10ml) for *Vitex negundo.*

No zone of inhibition was noted in most of the concentrations. Antibacterial activity of ethanol extract of medicinal plant against *S.aureus* represented in Table 8. Maximum zone of inhibition(17mm) was observed in 40µl concentrations of 50mg/1ml for *A.indica*, followed by 16mm was noted in *Az.indica*.

Effect of DMSO solvent on bacterial pathogens

Antibacterial activity of DMSO extract of medicinal plants against *P.aeruginosa* represented in Table 9. The highest zone of inhibition(27mm) was noted in the concentration of 100µl for *A.indica* followed by 26mm was noted in 10µl and 50µlfor *Calotropis procera* and *Vitex negundo* respectively. Antibacterial activity of DMSO extract of medicinal plants against *E.coli* represented in Table 10. Highest zone of inhibition(37mm) was noted in the concentration of 100µl for *A.vasica* followed by 22mm observed for *Vitex negundo*. Antibacterial activity of DMSO extract of medicinal plants against *K.pneumoniae* represented in Table 11.

The highest zone of inhibition (28mm) was noted in the concentration of 100µl for *Adhatoda vasica* followed by 27mm was noted in same concentration for *Acalypha indica*. Antibacterial activity of DMSO extract of medicinal plants against *S.pneumoniae* represented in Table 12. For *Acorus calamus*, the highest zone of inhibition about 23mm noted in the concentration of 100µl followed by 50µl concentration showed 22mm for *Ac.indica,* followed by for *Az. indica*, the 19mm was noted in 100µl. Antibacterial activity of DMSO extract of medicinal plants against the *S.aureus* represented in Table 13.

For *Azadirachta indica*, the highest zone of inhibition(22mm) was noted in the concentration of 100µl concentrationm followed by18mm noted in the concentration of 100µl for *C.procera*.

Effect of ethanolic extract of fungal pathogen

Antifungal activity of ethanol leaf extract of plant species against *Candida albicans* represented in Table 14. Highest zone of inhibition(24mm) was noted in the concentration of 10µl for *Az.indica* followed by 22mm was noted in the concentration of 100µl. 10µl concentration showed no sensitivity to the isolated *Candida albicans*.

Effect of DMSO solvent on fungal pathogen

Antifungal activity of DMSO leaf extract of plant species against *Candida albicans* represented in Table 15. Maximal zone of inhibition about 26mm was observed in 100µl for *Az.indica.* followed by 25mm was noted in 100µl for *A.calamus*.

PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS

Table 16 represented the phytochemical analysis of medicinal plants. Those plants provided positive results for Saponins, Tannins, Alkaloids, Flavanoids, Phenolic compounds and so on.

DISCUSSION

Infectious diseases are the leading cause of death across the world. As a global concern the antibiotic resistance by pathogens has emerged. Many of the antibiotics have been out of use as multidrug resistant pathogens have emerged [11].

Administered orally, the antibacterial compounds of herbs may be able to control wide range of microorganisms. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by the phytochemical constituents isolated from the medicinal plants listed in this study [12]. They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects [13].

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. Therefore, much attention has been given to traditional medicine in order to look for new leads to develop better drugs to treat resistant bacteria[14]. The present study indicates that the plant contains antimicrobial compound which can be further developed as phytomedicine for the therapy of infection[15].

Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development.

Table -1: Isolated Pathogens from HIV Postive Patients

Pathogens Isolated	Percentage	CD_4 COUNT/µL
Pseudomonas aeruginosa	30	300-380
Escherichia coli	24	350-470
Klebsiella pneumoniae	12	480-530
Streptococcus pneumoniae	6	500-670
Staphylococcus aureus	6	300-380
Candida albicans	18	250-400
TOTAL	92	

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Table -3: Morphological Characters of Isolated Fungi

Isolated fungal spp.,	Morphology
Candida albicans	Produced moist opaque creamy colony on blood agar. Clusters of round blastoconidia are present at some septae. Thick walled chlamydospores seen.

Table -2: Morphology, Staining and Biochemical charcteristics of isolated bacteria

Name of the Organism	Gram staining	Morphology	Motility	Biochemical tests								
				Indole	MR	VP	Citrate	Urease	Nitrate reduction test	H_2S Production Test	Carbohydrate fermentation test
P. aeruginosa	_	Bacilli	+	_	_	_	+	_	+	+	_
E.coli	_	Bacilli	+	+	+	_	_	_	+	_	+(A,G)
K. pneumoniae	_	Bacilli	+	_	_	+	+	+	+	_	+(A,G)
S.pneumoniae	+	Cocci	-	-	+	-	-	-	+	-	+(A,G)
S. typhi	_	Bacilli	+	_	+	_	+	_	+	_	+(A,G)
S. aureus	+	Cocci	_	_	+	_	_	+	+	_	+(A)

K. pneumoniae =Klebsiella pneumoniae; E.coli = Escherichia coli ; *S.aureus = Staphylococcus aureus; P.aeruginosa=Pseudomonas aeruginosa;S.typhi=Salmonella typhi*

Table -4: Antimicrobial Activity of Ethanol Extracts of Medicinal Plants Against *Pseudomonas Aeruginosa*

Medicinal plant	Part of the plant	Zone of inhibition(mm)								
		Extract amount (µl)								
		(10mg/10ml) 10-100mg			(10mg/1ml) 100-1000mg			(50mg/1ml) 1000-2000mg		
		10	50	100	25	50	100	25	30	40
Acalypha indica	**Leaf**	**08**	**16**	**16**	**10**	**08**	**12**	**10**	**11**	**10**
Acorus calamus	**Leaf**	**13**	**14**	**12**	**10**	**06**	**09**	**13**	**12**	**15**
Adhatoda vasica	**Leaf**	**11**	**12**	**13**	**14**	**22**	**12**	**21**	**19**	**27**
Azadirachta indica	**Leaf**	**18**	**20**	**25**	**20**	**24**	**23**	**22**	**21**	**20**
Calotropis procera	**Root**	**12**	**13**	**15**	**08**	**11**	**10**	**08**	**07**	**10**
Vitex negundo	**Leaf**	**08**	**11**	**12**	**12**	**16**	**14**	**20**	**15**	**22**

Table -5: Antimicrobial Activity of Ethanol Extracts Of Medicinal Plants Against *Escherichia coli*

Medicinal plant	Part of the plant	Zone of inhibition(mm)								
		Extract amount (µl)								
		(10mg/10ml) 10-100mg			(10mg/1ml) 100-1000mg			(50mg/1ml) 1000-2000mg		
		10	50	100	25	50	100	25	30	40
Acalypha indica	Leaf	-	-	26	-	-	23	-	-	-
Acorus calamus	Leaf	14	11	13	-	-	-	12	18	11
Adhatoda vasica	Leaf	8	8	14	10	8	12	-	-	-
Azadirachta indica	Leaf	-	10	10	8	11	18	7	-	10
Calotropis procera	Root	-	-	13	-	-	11	-	-	-
Vitex negundo	Leaf	8	14	16	10	12	19	10	17	16

Table -6: Antimicrobial Activity of Ethanol Extracts of Medicinal Plants Against *Klebsiella pneumoniae*

Medicinal plant	Part of the plant	Zone of inhibition(mm)								
		Extract amount (µl)								
		(10mg/10ml) 10-100mg			(10mg/1ml) 100-1000mg			(50mg/1ml) 1000-2000mg		
		10	50	100	25	50	100	25	30	40
Acalypha indica	Leaf	-	15	20	-	-	12	-	-	-
Acorus calamus	Leaf	7	9	15	-	12	16	-	-	21
Adhatoda vasica	Leaf	5	8	14	11	12	18	12	-	-
Azadirachta indica	Leaf	13	11	14	19	15	18	-	12	16
Calotropis procera	Root	21	11	18	15	10	13	-	-	-
Vitex negundo	Leaf	18	10	14	14	15	12	16	-	-

Table -7: Antimicrobial Activity of Ethanol Extracts of Medicinal Plants Against *Streptococcus pneumoniuae*

Medicinal plant	Part of the plant	Zone of inhibition(mm)								
		Extract amount (µl)								
		(10mg/10ml) 10-100mg			(10mg/1ml) 100-1000mg			(50mg/1ml) 1000-2000mg		
		10	50	100	25	50	100	25	30	40
Acalypha indica	Leaf	-	-	14	-	-	14	-	-	-
Acorus calamus	Leaf	-	10	15	12	14	16	14	14	12
Adhatoda vasica	Leaf	-	-	-	-	-	-	8	10	-
Azadirachta indica	Leaf	5	9	10	12	9	14	-	-	-
Calotropis procera	Root	-	-	-	-	-	-	-	-	-
Vitex negundo	Leaf	-	-	18	-	-	12	-	-	-

Table -8: Antimicrobial Activity of Ethanol Extracts of Medicinal Plants against *Staphylococcus aureus*

Medicinal plant	Part of the plant	Zone of inhibition(mm)								
		Extract amount (µl)								
		(10mg/10ml) 10-100mg			(10mg/1ml) 100-1000mg			(50mg/1ml) 1000-2000mg		
		10	50	100	25	50	100	25	30	40
Acalypha indica	Leaf	11	8	12	-	-	12	-	-	17
Acorus calamus	Leaf	10	11	12	-	-	14	-	-	15
Adhatoda vasica	Leaf	8	10	14	-	-	14	-	-	6
Azadirachta indica	Leaf	9	12	16	-	-	15	-	-	8
Calotropis procera	Root	10	12	12	-	-	12	-	-	10
Vitex negundo	Leaf	12	13	13	-	-	16	-	-	10

Table -9: Antimicrobial Activity of DMSO Extracts of Medicinal Plants Against *Pseudomonas aeruginosa*

Name of Medicinal plant	Part of Plant used	Zone of inhibition (mm) Extract amount 10-100mg (10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	-	11	27
Acorus calamus	Leaf	-	9	18
Adhatoda vasica	Leaf	-	12	23
Azadirachta indica	Leaf	19	22	17
Calotropis procera	Root	26	10	15
Vitex negundo	Leaf	12	26	13

Table -10: Antimicrobial Activity of DMSO Extracts of Medicinal Plants Against *Escherichia coli*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm) Extract amount 10-100mg (10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	-	16	18
Acorus calamus	Leaf	-	9	-
Adhatoda vasica	Leaf	-	18	37
Azadirachta indica	Leaf	18	12	18
Calotropis procera	Root	16	9	-
Vitex negundo	Leaf	22	5	12

Table -11: Antimicrobial Activity of Dmso Extracts Of Medicinal Plants Against *Klebsiella pneumoniae*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm) Extract amount 10-100mg (10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	-	24	27
Acorus calamus	Leaf	-	21	-
Adhatoda vasica	Leaf	-	13	28
Azadirachta indica	Leaf	-	22	24
Calotropis procera	Root	-	-	14
Vitex negundo	Leaf	9	12	-

Table -12: Antimicrobial Activity of Dmso Extracts Of Medicinal Plants Against *Streptococcus pneumoniuae*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm) Extract amount 10-100mg (10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	-	22	-
Acorus calamus	Leaf	-	18	23
Adhatoda vasica	Leaf	-	14	18
Azadirachta indica	Leaf	-	9	19
Calotropis procera	Root	18	14	14
Vitex negundo	Leaf	21	17	14

Table -13: Antimicrobial Activity of Dmso Extracts Of Medicinal Plants Against *Staphylococcus aureus*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm) Extract amount 10-100mg(10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	12	10	14
Acorus calamus	Leaf	8	9	12
Adhatoda vasica	Leaf	5	9	14
Azadirachta indica	Leaf	12	16	22
Calotropis procera	Root	14	17	18
Vitex negundo	Leaf	14	12	15

Table -14: Antifungal Activity of Ethanolic Extract of Medicinal Plants Against C*andida albicans*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm)		
		Extract amount 10-100mg(10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	21	16	22
Acorus calamus	Leaf	18	15	20
Adhatoda vasica	Leaf	18	15	21
Azadirachta indica	Leaf	24	18	22
Calotropis procera	Root	21	18	14
Vitex negundo	Leaf	0	16	17

Table -15: Antifungal Activity of Ethanolic Extract of Medicinal Plants Against *Candida albicans*

Name of Medicinal plant	Part of plant used	Zone of inhibition (mm)		
		Extract amount 10-100mg(10mg/10ml)		
		10µl	50µl	100µl
Acalypha indica	Leaf	23	21	18
Acorus calamus	Leaf	15	23	25
Adhatoda vasica	Leaf	16	15	20
Azadirachta indica	Leaf	22	19	26
Calotropis procera	Root	21	23	21
Vitex negundo	Leaf	18	13	22

Table -16: Phytochemical Screening of the Stem Methanol Extract of *Tylophora indica* L

S. No.	Phytochemical analysed	*A.indica*	*A.calamus*	*A.vasica*	*A.indica*	*C.procera*	*V.negundo*
1.	Tannins	+	+	+	-	-	-
2.	Saponins	+	-	+	+	-	+
3.	Flavonoids	+	+	+	-	-	+
4.	Terpenoids	-	-	-	-	-	+
5.	Glycosides	+	-	-	-	-	-
6.	Phytosterols Libermann Burchard's test	+	+	+	+	+	+
7.	Phenolic compounds i) Ferric Chloride ii)Gelatin	- -	- -	- -	+ -	- -	+ -
8.	Alkaloids i) Mayer's test ii) Wagner's test iii) Hagner's test	- + -	- + -	- + -	- + -	- + -	- + -
9.	Carbohydrates i) Molisch's test ii)Fehling test iii)Benedict's test	+ - -	+ - -	+ - -	+ - -	+ - -	+ - -

\+ ---- Positive - ---- Negative

A.indica------- Acalypha indica, A.calamus---- Acorus calamus, A.vasica------- Adhatoda vasica, A.indica------- Azadirachta indica
C.procera----- Calotropis procera, V.negundo---- Vitex negundo