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ANTI-MICROBIAL ACTIVITY OF *Ixora alba*, *Plumeria obtusa* AND *Psidium guajava*

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Abstract- Studies on the antimicrobial activities of medicinal plants have clearly become a progressive trend. With advances in laboratory techniques, renewed interest in the field and the scientific validation of the traditional use, the possibility now exists to bring traditional medicine to such a level of recognition that it becomes an accepted alternate regimen to western healthcare systems. Due to the indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. It appears that the real need for the future will be agents to treat the drug-resistant Gram-negative pathogens. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One of the approaches is to screen local medicinal plants for possible antimicrobial properties. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants. After a detailed literature survey of Ayurvedic texts and published research articles, for present study, 3 Indian medicinal plants belonging to the families Apocynaceae (*Plumeria obtusa)*, Rubiaceae (*Ixora alba*) and Myrtaceae (*Psidium guajava*) were shortlisted for the present study. These were screened for their antimicrobial properties against pathogens including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Escherichia coli*, *Shigella dysenteriae, Vibrio cholerae*, *Haemophilus parahaemolyticus, Proteus mirabilis, Proteus vulgaris, Cryptococcus neoformans* and *Candida albicans*.

Keywords- Antimicrobial activity, Apocynaceae, Rubiaceae, Myrtaceae, herbal antibiotic

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Introduction

Infectious diseases continue to be a major challenge in the South East Asia (SEA) Region. They are estimated to be responsible for about 40% of the 14 million deaths annually in the Region and account for 28% of the global burden of infectious diseases. Of 350 million lives that are lost due to communicable diseases globally, SEA Region accounts for 89 million. The brunt is mainly borne by children, women and marginalized sections of society. Children show greater vulnerability. Infectious diseases represent 7 out of 10 top causes of child deaths in developing countries, and account for nearly 60% of all such deaths"[1].

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the 'antibiotic era' barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens [2]. Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects [3].

About 80 % of the world population relies on botanical preparations as medicines to meet their health needs [4,5]. In recent years there has been a growing interest to evaluate plants possessing antimicrobial activity. One approach is to screen local medicinal plants for possible antimicrobial properties. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained.

Role of medicinal plants is dual in the development of new drugs:

- They may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or;
- A phytomedicine to be used for the treatment of disease

Each of these plants of genus *Ixora*, *Plumeria* and *Psidium* has been reported to have medicinal value in terms of anti-inflammatory, anticancer, antidiarrhoeal or antibacterial activity. It was therefore decided to study the antimicrobial activity of hot methanolic extracts of barks of *I. alba* and *P. obtusa,* and leaves of *P. guajava* plants, *in vitro*, by using various pathogenic bacteria.

Review of Literature

The flowers of *I. coccinea* Linn. have been used in traditional Indian systems of medicine for dysentery, healing of ulcers and, also, for an anti-tumour activity. Reports are available on antibacterial effect of ether and methanolic extracts of leaves of *I. coccinea* Linn. [6] and bark powder of *I. brachiata* Roxb., [7] but no reports were found on the antibacterial activity of methanolic extract of bark of *I. alba.*

Methanolic extracts of *P. acuminata* as well as *P. alba* have been studied for their antimicrobial activity [8]. It is also reported to be used in asthma, malaria, gonorrhea, fever, and as a cathartic, uretic and haemogogue [9]. Antimicrobial activity of essential oils present in its flowers have been reported [10].

Antibacterial effect of methanolic extract of leaves of *Psidium guajava* has been studied by many scientists. *Psidium guajava* sprout extract (young leaves of *P. guajava*) showed antibacterial activity against *S. aureus* and *E. coli* and complete inhibition of *S. epidermidis* and *S. typhimurium* [11,12]. *P. guajava* extracts have been tested for antimicrobial activities against different species of diarrhoeagenic *Salmonella*, *Shigella*, and also *E. coli* [13]. Guava sprouts have been used in Brazilian medicine for gastrointestinal disorders, the antimicrobial activity of *P. guajava* showed inhibition of *E. coli* and *S. aureus, S. typhi, S. flexneri* and *S. dysenteriae* [14,15]. The work was also reported for *P. guajava* extracts against dermatophytes *Trichophtyon tonsurans, Trichophyton rubum, and Microsporum fulvum* [16]. Extracts from the bark of *P. guajava* were found to be more efficient than leaf extracts in inhibiting the dermatophytes [17-20]. Anticough and antimicrobial activities of *P. guajava* leaf extracts; showed that phytomedicines are effective in treating a number of infectious diseases [21]. Numerous publications have documented the antibacterial and antifungal potency of flavonoids [22-24]. This activity was attributed mostly to the ability of flavonoids to complex with bacterial cell wall and therefore, inhibiting the microbial growth [23].

Material and Methods

Twigs *I. alba* and *P. obtusa,* and *P. guajava* were collected and mounted on Herbarium. These were identified from BSI (Botanical survey of India) Herbarium. Plant extracts were prepared from bark of *I. alba* and *P. obtusa,* and leaves of *P. guajava* using soxhlet apparatus (methanol) The dried plant extracts (Hot methanolic extract - HME) were reconstituted in methanol up to a concentration of 50 mg/ml during the present study. In order to screen and determine antibacterial effects of selected plant extracts and phytochemicals, 18 organisms representing different groups were used. The selection of organisms was mainly based on their reported pathogenic activities. The cultures of bacteria were maintained on appropriate agar slants, and stored at 4°C till use throughout the study and used as stock cultures.

During the present investigations dehydrated culture media, procured from Hi-media Laboratories Pvt Limited, Mumbai were used. The agar-cup method was found to be the suitable diffusion technique for testing aqueous suspensions of plant extracts.

Minimum Inhibitory Concentrations (MIC) is the least amount of antimicrobial that will inhibit growth of an organism after overnight incubation. The MICs are mainly used to determine antibiotic sensitivities of infectious organisms. MICs are used as reference points in the evaluation and comparison of new and existing antimicrobial agents.

There exist different ways of expressing the biological activity of plant extracts based on the technique used. The agar diffusion method led to results being given in terms of diameter of the inhibition zone (mm) while the micro-dilution method yield MIC values, i.e. the minimum concentration at which inhibition is observed (mg/ml).

Results

The [Table-1] summarizes the results of the initial screening of individual extracts of selected plants at 50 mg/ ml concentration and when used in combination using Agar cup diffusion method [Plates-1](a-r). The HME of bark of *I. alba* was found to be very effective against almost all the pathogens tested except against *S. pyogenes.*

Table 1- Antimicrobial activities of Hot methanolic extracts (50 mg / ml) to determine of selected plants using the agar diffusion method.

List of microorganisms	Zone of Inhibition (diameter in mm)				
	Control (Methanol)	*Ixora alba*	*Plumeria obtusa*	*Psidium guajava*	Combination
S. aureus NTCC 3750	NI	18±0.80	16±0.41	22±0.49	20±0.67
S. aureus MTCC 96	NI	14±0.66	14±0.49	17±0.41	17±0.42
S. aureus MTCC 25923	NI	15±0.88	15±0.32	20±0.49	19±0.42
MRSA Strain (Lab. isolate)	NI	16±0.49	14±0.42	20±0.63	20±0.20
P. aeruginosa MTCC 1688	NI	14±0.41	14±0.41	16±0.67	18±0.82
P. aeruginosa MTCC 424	NI	21±0.63	14±0.80	24±0.49	25±0.76
E. coli MTCC 1687	NI	13±0.52	14±0.66	14.5±0.32	17±0.49
E. coli MTCC 10148	NI	14±0.63	13±0.75	18±0.63	15±0.41
P. mirabilis (Lab. isolate)	NI	14±0.20	16±0.41	19±0.41	19±0.20
P. vulgaris (Lab. isolate)	NI	14±0.32	18±0.63	22±0.63	17±0.63
S. typhi MTCC 733	NI	17±0.61	18±0.20	16±0.63	19±0.84
K. pneumoniae MTCC 109	NI	14±0.80	15±0.61	18±0.32	18±0.82
S. dysenteriae (Lab. isolate)	NI	15±0.67	17±0.55	24±0.71	24±0.54
S. pyogenes (Lab. isolate)	NI	13±0.71	14±0.20	19±0.32	25±0.61
V. cholerae (Lab. isolate)	NI	13±0.20	15±0.80	19±0.32	20±0.80
H. parahaemolyticus (MTCC 1776)	NI	20±0.84	19±0.32	26±0.32	21±0.42
C. albicans MTCC 183	12±0.63	32±0.20	16±0.26	14±0.42	17±0.88
C. neoformans NCIM 3542	12±0.75	37±0.52	20±0.49	13±0.20	21±0.61

NI- No inhibition; Values are mean±SD of three experiments in triplicate.

The HME of bark of *P. obtusa* was found to be effective against a majority of the bacteria tested, but it exerted a very limited effect on *E. coli, P. vulgaris* and *K. pneumoniae.* The HME of leaves of *P. guajava* was found to be effective against all the bacteria tested but had little effect on *C. albicans,* and *C. neoformans.*

A combination of the HME of the bark of *P. obtusa* and the leaf extract of *P. guajava*, (1:1), was found to be effective against all the pathogens tested.

These results are also presented in the graphical representation in [Fig-1] and [Fig-2].

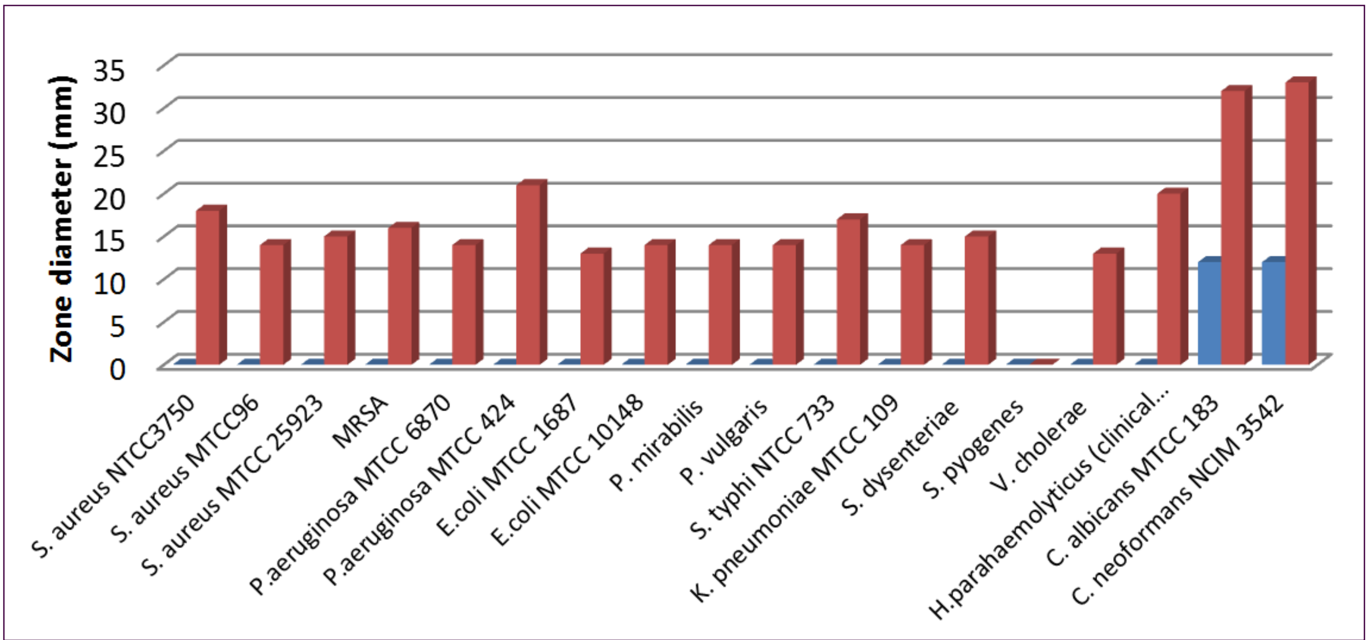


Fig. 1- Antimicrobial activity of HME of *I. alba* compared with methanol as control at 50 mg/ml against various pathogens

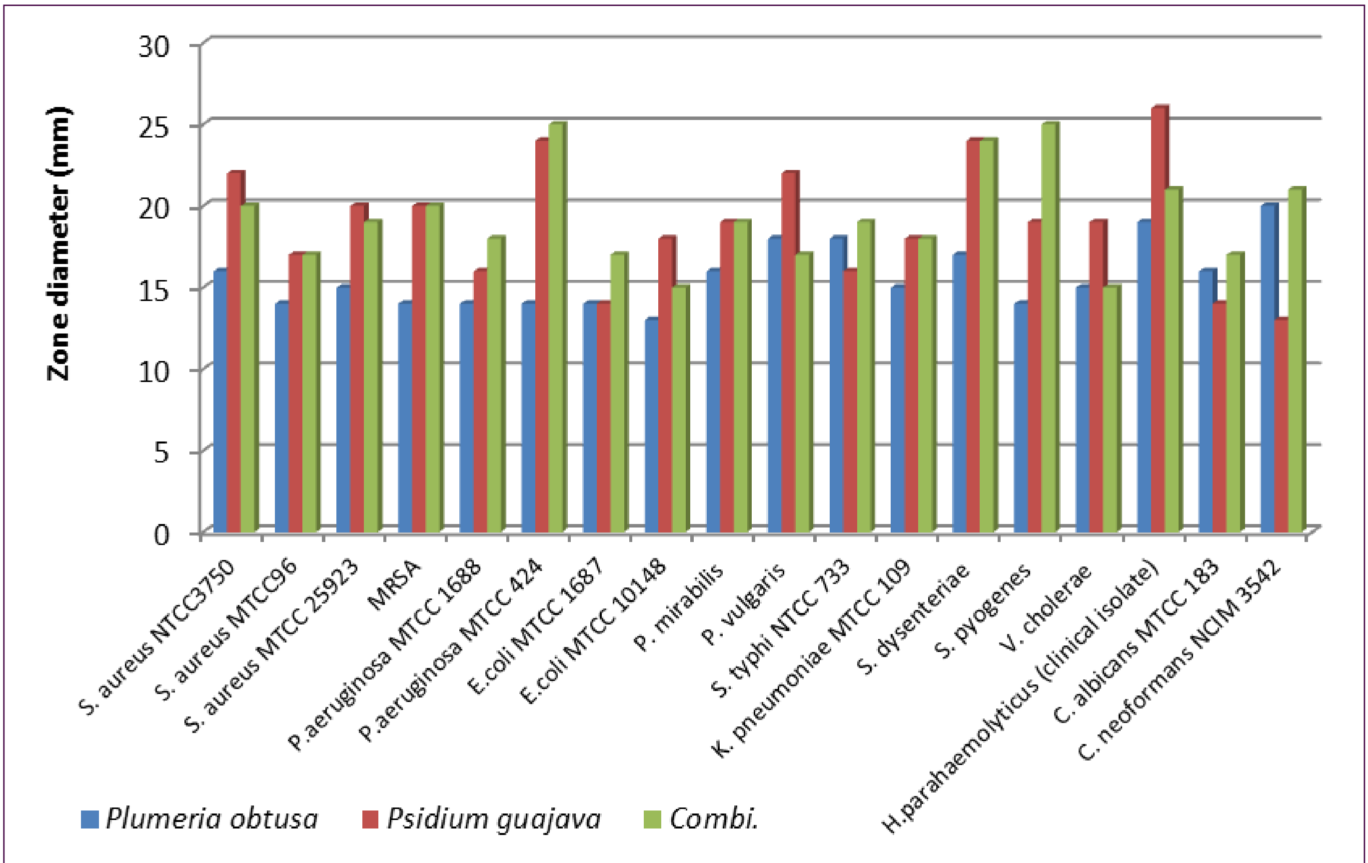
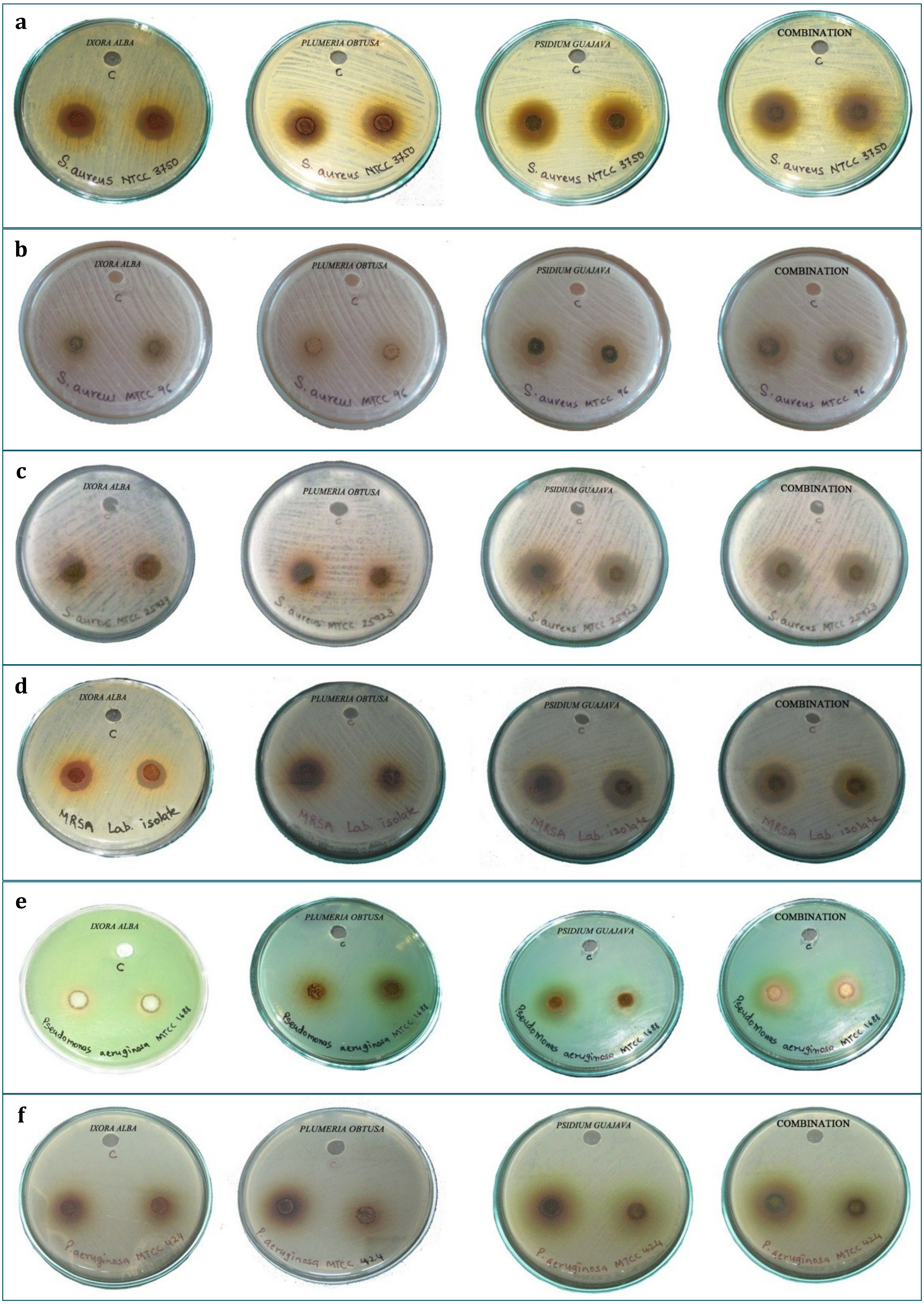
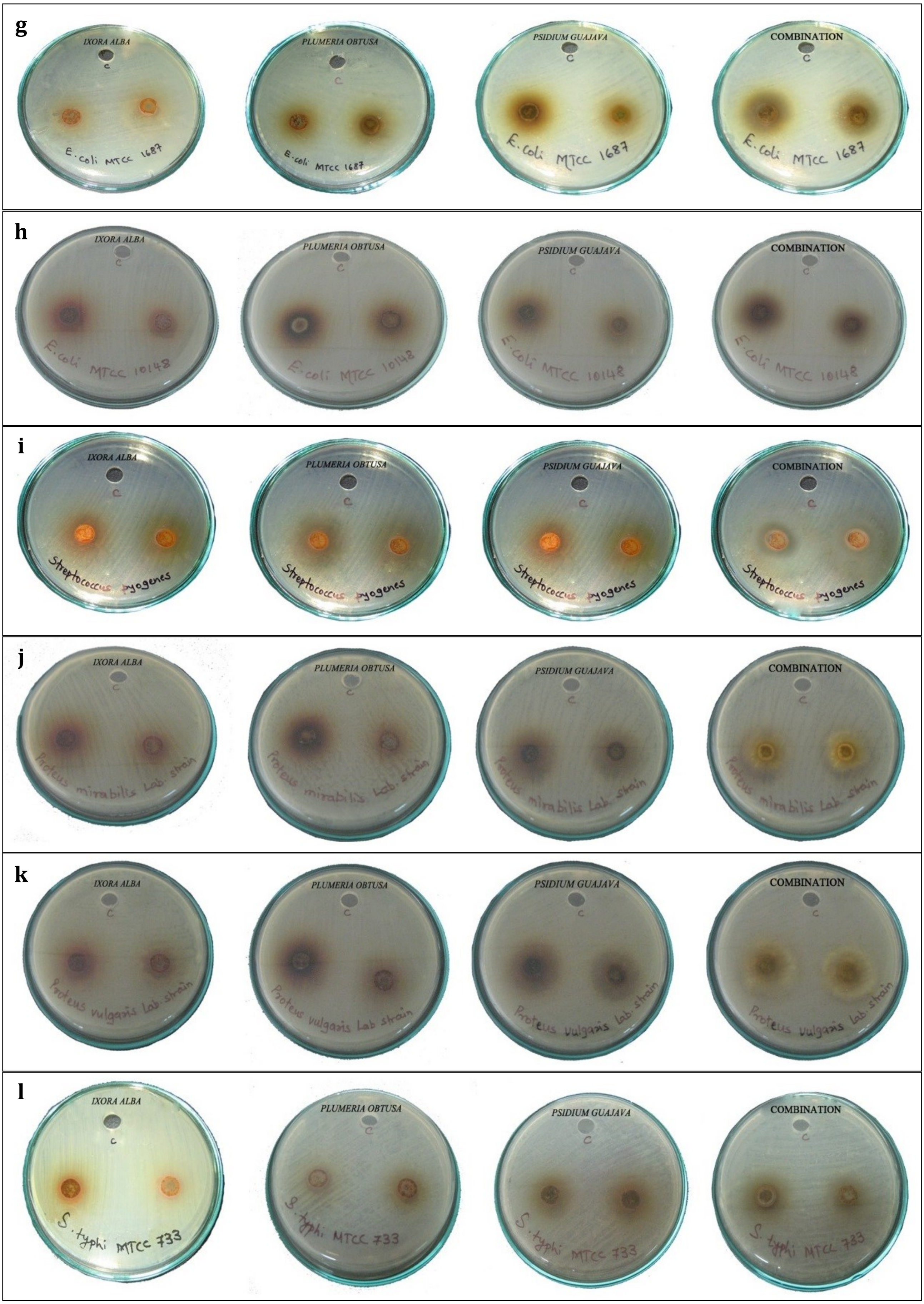
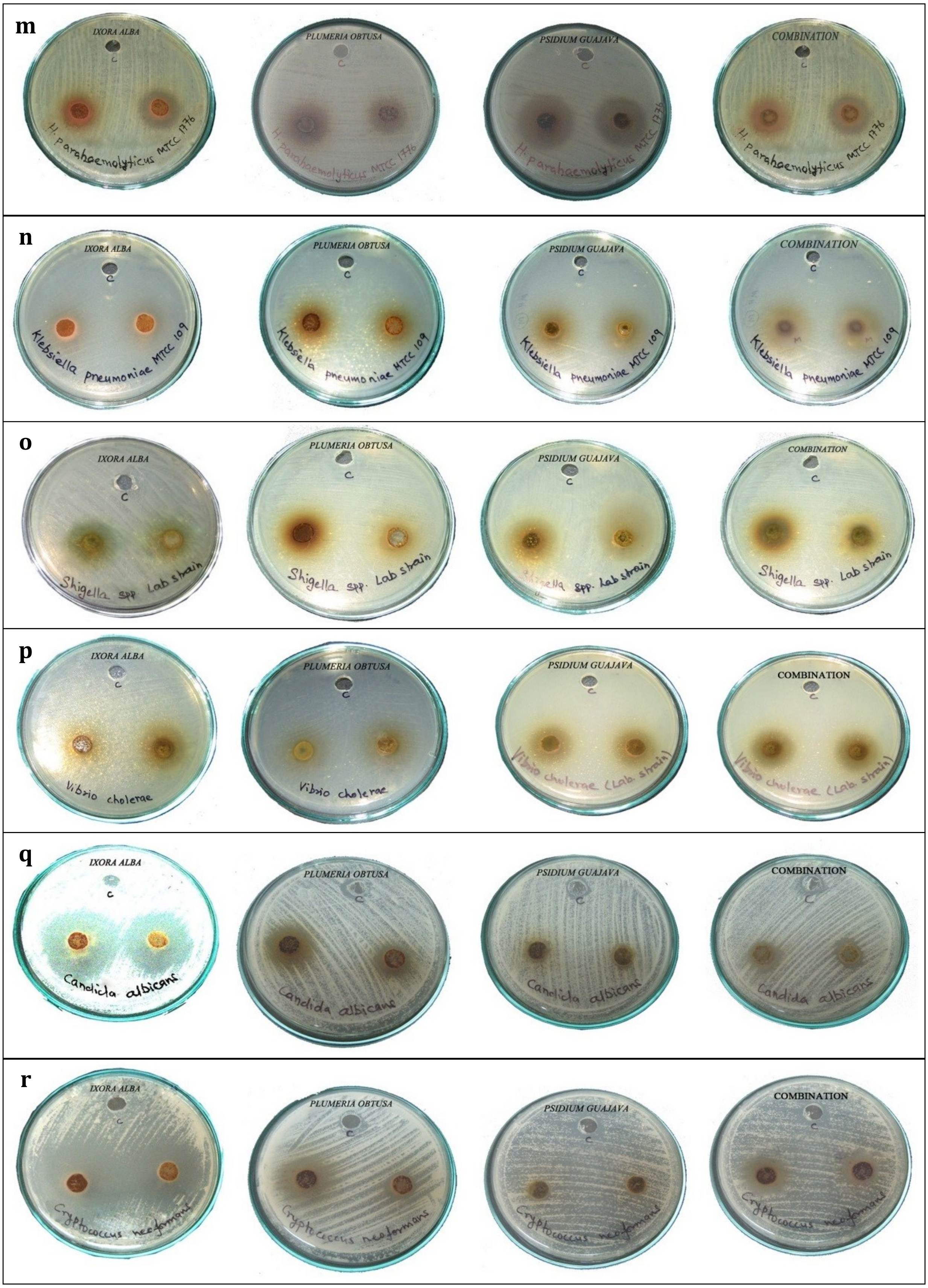


Fig. 2- Antimicrobial activity of HME of *P. obtusa* and *P. guajava* compared with combination of their extracts, (1:1) at 50 mg/ml against various pathogens


a
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
S. aureus NTCC 3750
b
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
S. aureus MTCC 96
c
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
S. aureus MTCC 25923
d
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
MRSA Lab. isolate
e
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
Pseudomonas aeruginosa MTCC 1688
f
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
P. aeruginosa MTCC 424


g
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
E. coli MTCC 1687
h
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
E. coli MTCC 10148
i
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
Streptococcus pyogenes
j
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
Proteus mirabilis Lab. strain
k
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
Proteus vulgaris Lab. strain
l
IXORA ALBA
PLUMERIA OBTUSA
PSIDIUM GUAJAVA
COMBINATION
C
S. typhi MTCC 733



Plates 1(a-r)- Antifungal activity of hot methanolic extracts of selected plants against *S. aureus* (MTCC 3750), *S. aureus* (MTCC 96), *S. aureus* (MTCC 25923), MRSA (lab. strain) *P. aeruginosa* (MTCC 1688) , *P. aeruginosa* (MTCC 424), *E. coli* (MTCC 1687), *E. coli* (MTCC 10148), *S. pyogenes* (lab. strain), *P. mirabilis* (lab. strain), *P. vulgaris* (lab. strain), *S. typhi* (MTCC 733), *H. parahaemolytic* (MTCC) , *K. pneumoniae* (MTCC 109), *S. dysenteriae* (lab. Strain), *V. Cholera* (lab. Strain), *C. albicans* (MTCC 183), *C. neoformas* (NCIM 3542) respectively.

MIC values of the selected plant extracts using the broth dilution method are presented in [Table-2]. The results shown in this table are based on the presence of growth or no growth on the plate.

Table 2- Screening of Hot methanolic extracts to determine MIC values of the extracts of the selected plants using the broth dilution method.

List of microorganisms	MIC Values (mg/ml)		
	Ixora alba	*Plumeria obtusa*	*Psidium guajava*
S. aureus NTCC 3750	10	6	0.5
S. aureus MTCC 96	10	5	0.6
S. aureus MTCC 25923	30	20	3
MRSA Strain (lab. isolate)	6	2	2
P. aeruginosa MTCC 1688	2	6	0.5
P. aeruginosa MTCC 424	8	8	2
E. coli MTCC 1687	20	15	6
E. coli MTCC 10148	6	7	4
P. mirabilis (lab. isolate)	9	10	2
P. vulgaris (lab. isolate)	5	20	4
S. typhi MTCC 733	3	3	0.5
K. pneumoniae MTCC 109	7	6	1
S. dysenteriae (lab. isolate)	5	4	2
V. cholerae (lab. isolate)	4	20	3
H. parahaemolyticus (MTCC 1776)	4	9	0.6
C. albicans MTCC 183	0.7	4	10
C. neoformans NCIM 3542	0.6	2	4

Discussion

[Table-3] records total antimicrobial potency of the hot methanolic extract of the selected plants in terms of percentage activity. From the table it can be observed that the total antimicrobial potency of *I. alba, P. obtusa* and *P. guajava* was found to be 88.89 %, 94.44 % and 94.44 %, respectively. The combination of extracts of *P. obtusa* and *P. guajava* proved to be most effective as the total antimicrobial potency was found to be 100%.

Percent activity values recorded, give further support to the folkloric use of these plants in the treatment of infectious diseases as the values in general were found to be above 85% (Table 4). Hot methanolic extract of the combination of, *Plumeria obtusa* and *Psidium guajava*, 1:1 in particular showed notable efficiency (100% activity) against some of the bacterial strains used. Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites [25].

In the present study, the results indicate that the combined effect of the plant extracts have an antibacterial enhancement (synergistic effects) against some of the pathogenic bacteria tested viz, *P. aeruginosa, S. typhi, E. coli, S. pyogenes* and the fungi, *C. albicans* and *C. neoformans*.

Table 3- Antimicrobial potency of the extracts of the selected plants in terms of percent activity.

/Plants	Percent activity (%)
Ixora alba	88.89
Plumeria obtuse	94.44
Psidium guajava	94.44
Combination *Plumeria obtusa* + *Psidium guajava*, 1:1	100

Bacterial Susceptibility Index (BSI) values (Table 4) are useful in evaluating the susceptibility of the different strains of bacteria towards the plant extracts being investigated. Amongst the organisms tested during the present study, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *P. vulgaris*, *S. typhi*, *K. pneumoniae*, *S. dysenteriae*, *H. parahaemolyticus* and *C. albicans* were found to be susceptible to all the hot methanolic extracts of the plants investigated (100% susceptibility) on the other hand *E. coli*, *V. cholerae* and *C. neoformans* were found to have a susceptibility index at 75%.

Table 4- Screening for Bacterial susceptibility index, BSI

List of microorganisms	BSI
S. aureus NTCC 3750	100
S. aureus MTCC 96	100
S. aureus MTCC 25923	100
MRSA Strain (lab. isolate)	100
P. aeruginosa MTCC 1688	100
P. aeruginosa MTCC 424	100
E. coli MTCC 1687	75
E. coli MTCC 10148	75
P. mirabilis (lab. isolate)	100
P. vulgaris (clinical isolate)	100
S. typhi MTCC 773	100
K. pneumoniae MTCC 109	100
S. dysenteriae (lab. isolate)	100
V. cholerae (lab. isolate)	75
H. parahaemolyticus (MTCC 1776)	100
C. albicans MTCC 183	100
C. neoformans NCIM 3542	75

Conclusion

The results of this study have clearly indicated that HME of the bark of extract of *I. alba* have shown a very significant antifungal activity compared to antibacterial activity. Hot methanolic bark extract of *P. obtusa* shows promising antimicrobial activity whereas hot methanolic leaf extract of *P. guajava* exhibited much better antibacterial activity as against antifungal activity.

An important observation made during the present study was that all the three plant extracts are highly effective against *P. aeruginosa*, an important Gram negative pathogen as well as *S. aureus* an important Gram positive pathogen. This can be considered as an important observation as *P. aeruginosa*, an opportunistic pathogen and *S. aureus* have been found to be resistant to a variety of known antibiotics. On the basis of the results obtained, it can be concluded that the crude extracts of all the selected plants used in the present study exhibit good antimicrobial activity against both Gram positive as well as Gram negative organisms, indicating these extracts are broad spectrum in nature.

Conflicts of Interest: None declared.

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