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In-vitro Experimental Studies on Latex yielding Plants for their Antimicrobial activity.

Satyaveni G, K Rishitha, M Raj Kumar, Md Rameezuddin, S Asha, and R Bharath Kumar*.

School of Biotechnology, Vignan's University, Vadlamudi, Guntur - 522213, Andhra Pradesh, India.

ABSTRACT

The present work will focus on the importance of plant base latex and their antifungal and antibacterial activity. The plants were selected for Extracts to test their broad spectrum of anti microbial activity resistance against for the selected plant Latex as per the literature sources. There are about 7 indigenous Latex yielding plants were selected which are having diversified uses like medicinal, economic, timber and fibre yielding species like *Calotropis gigantea* (Jilledu),*Carica papaya* (papaya), *Plumeria alba*, *Ficus religiosa*(Raavi), *Ficus bengalensis* (Banyan), *Euphorbia milli* (crown of throns), *Acharas zapota* (Sapota) viz.of Guntur region (Vadlamudi, Tenali etc.). The Anti-fungal activity and the anti bacterial activity were tested against 7 plant latex samples. A total number of 92 plant extracts (latex samples) were prepared as an individual of 28 ethanol, methanol, acetone and hot water extracts belongs to the 7 individual plant species and another set of 6 plant extracts were prepared in combination of ethanol, methanol, acetone & n-butanol.7 Plant extracts were prepared in multiple combinations ethanol, methanol, acetone, n-butanol. All these plant extracts were subjected against their antibacterial and anti fungal screening analysis, out of these plant extracts (individual, combination & multiple) 5 plant extracts have been expressed the cognizable zone of expression i.e. 25 mm > 30 mm inhibition. Therefore these combinations were suggested for further analysis of latex samples for their antimicrobial activity.

Keywords: In-vitro studies, selected latex plants, antimicrobial activity.

Corresponding author

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INTRODUCTION

Vignan's University (VU) (formerly Vignan's Engineering College is a premier institution affiliated to Jawaharlal Nehru Technological University in Andhra Pradesh). It is having the splendid avenue, imposing buildings and sprawling playgrounds, and the verdure in and around the campus. The college is a virtual haven of rural quiet and idyllic beauty. Since its inception in 1997, VU has been striving to promote high quality standards in technical education & research for the aspirants of Engineering Studies.

Topography

Vignan's University is located in the serene environs of Vadlamudi on the Guntur- Tenali highway, about 14 km from Guntur and 11 km from Tenali. The nearest railway station Tenali is located on Chennai – Kolkata trunk line.

Brief enumeration and description of habit & habitat of selected latex yielding plant species for their antimicrobial activity:

Acharas zapota (L)P.Royen **(Sapotaceae)**
Ln: Sapota

Distribution & Uses:

Trees. Cultivated in India for its fruit value, while in South-East Mexico, Guatemala and other countries it is commercially grown for the production of chickle which is a gum like substance obtained from latex and is mainly used for preparation[1,6]. Chicle and gutta parcha are extracted from its latex[1,4]. These are used as a base material in chewing gum and in some other industrial products. Fruit & Bark-Febrifuge, Seed–Diuretic. The fruit is a good source of digestible sugar (12-18%) and an appreciable source of protein, fat fibre, minerals, Calcium and Iron[1,2].

Calotropis gigantea L. W.Ait. **(Apocynaceae)**
Ln.: Jillaedu, milkweed or swallow-wort

Distribution & Uses:

Woody Shrubs. It is native to Cambodia, Indonesia, Malaysia, Philippines,Thailand, Sri Lanka, India and China. It is used as a traditional medicinal plant with unique properties, to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea[1,5]. The leaves and latex of *C.gigantea* is having excellent fungicidal properties[4,6,17].

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Carica Papaya L. **(Caricaceae)**
Ln: Papaya

Distribution &Uses:

Trees. Native to the tropics of the Americas, then distributed to southern Mexico (Central America, and northern South America, the papaya is now cultivated in most tropical countries. Papaya fruit is a source of nutrients such as provitamin A, carotenoids, vitamin C, and dietary fiber[2,6]. Papaya skin, pulp and seeds also contain a variety of phytochemicals, including lycopene and polyphenols. The stem and bark may be used in rope production[1,6]

Euphorbia milli L. Des Moul. **(Euphorbiaceae)**
Ln: Crown of Thorns

Distribution & Uses:

Shrubs. Native to Madagascar,cultivated in settled areas in the Philippines,India, China & Brazil etc.The sap is moderately poisonous[11,14]. Leaf, Latex & flower consists the properties of antibacterial, antifungal & control of aflatoxins (*Aspergillus*)[1,5].

Ficus bengalensis L**. (Moraceae)**
Ln.: Marri, Indian Banyan Tree

Distribution & Uses:

Trees. Native to the subcontinent. Stem Bark is Astringent to bowels; useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy[4,7]. The aerial roots are useful in syphilis, biliousness, dysentery, inflammation of liver etc. The tree is considered sacred and used in worships and traditional ceremonies[6,15].

Ficus religiosa L**. (Moraceae)**
Ln.: Peepal Tree,The Bodhi Tree, Sacred Fig tree

Distribution & Uses:

Trees. Native to Nepal, India, Bangladesh, Myanmar, Pakistan, Sri Lanka region. It is used as a traditional medicinal plant with unique properties, to treat diseases such as asthma, diabetes, diarrhea,

epilepsy, gastric problems, inflammatory disorders, infectious and sexual disorders[4,16]. The tree is considered sacred and used in worships and traditional ceremonies[15,16,19].

Plumeria alba L. **(Apocynaceae)**
Ln.: Deva Kanchanam

Distribution & Uses:

Deciduous shrubs and small trees. Native to Central America, Mexico, the Caribbean, and South America as far south as Brazilbut have been spread throughout the world's tropics. *Plumeria* flowers are most fragrant and used in worships and traditional ceremonies[2,4]. Stem bark is used as an anti-inflammatory, antileprotic, antipruritis, antirheumatism and antimalarial agent. Also used in heat boils, fever, carbuncles, tumor, filariasis & microbial diseases[4,17].

MATERIALS AND METHODS

Vignan's University has campus with a good number of plants. It includes landscaping gardens, exotic elements and natural forest elements, includes rare and endemic categories of trees, shrubs, herbaceous members, climbers and a good number medicinal, latex yielding plants like *Acharas zapota* (Sapota),*Calotropis gigantea* (Jilledu), *Carica papaya* (papaya), *Euphorbia milli* (Crown of thorns), *Ficus bengalensis* (Banyan), *Ficus religiosa* (Raavi) and *Plumeria alba* (Deva Kanchanam). An inventory experimental studies were conducted on selected most promising plant species which are having utilization of domestic, commercial importance of plant based latex samples. Methodology was adopted for the above mentioned studies are as per standard literature sources[4,18].

The present work was conducted in School of Biotechnology, Microbiology lab Vignan's University, Vadlamudi to determine the antifungal and antibacterial activity of latex samples of *Acharas zapota* (Sapota),*Calotropis gigantea* (Jilledu), *Carica papaya* (papaya), *Euphorbia milli* (Crown of thorns), *Ficus bengalensis* (Banyan), *Ficus religiosa* (Raavi) and *Plumeria alba* (Deva Kanchanam) etc. Against two selected bacterial pathogens viz., *Bacillus cereus & E.coli* (bacterial species) and against three selected fungal pathogens viz*., Aspergillus niger, Cercospora pongamaie, Phytoptara infestans* (fungal species) for antimicrobial activities of plant latex extracts [2,4,18].

Preparation of latex samples:

The latex samples are collected from the selected plants of both in university campus and Vadlamudi environs of Guntur. The collected latex samples are dried crushed into fine powder using sterile mortar and pestle.

These fine powdered latex samples are subjected to successive solvent extraction. The extraction was performed using the following solvents acetone, methanol, ethanol & hot water respectively in the first stage of project i.e. just for preparation of latex samples. In further stages we used combinations of these solvents along with n-butanol.1 ml of Sample & 5 ml of solvent are taken in test tube and they are placed in orbital shaking incubator for about 24 hrs .After 24 hrs they are taken out from the orbital shaking incubator and tested for their solubility[16,18,27]. From a group of test tubes with Latex samples along with solvents the test samples are selected based on their maximum solubility of Latex. The combinations are as follows:

DUAL COMBINATIONS:	MULTIPLE COMBINATIONS:
Acetone + Ethanol (A+E)	Acetone + Ethanol + Methanol (A+E+M)
Ethanol + n-butanol (E+ n-bt)	Acetone + Ethanol + n-butanol (A+E+n-b)
Acetone + Methanol (A+M)	Acetone + Methanol + n-butanol (A+M+n-b)
Acetone + n-butanol (A+ n-bt)	Ethanol + Methanol+ n-butanol (E+M+n-b)
Methanol + Ethanol (M+E)	

Preparation of Media and Screening of Antimicrobial Activity:

Antimicrobial screening was done by the standard procedures described by [2,16] etc.

Media & Microorganisms:

The suitable culture media was prepared by dissolving the below mentioned ingredients for the respective microorganisms [3,18]. The contents were autoclaved at 15 lbs for 15 min. microorganisms taken are *Bacillus cereus & E.coli* (bacterial species) and *Aspergillus niger, Cercospora pongamaie, Phytoptara infestans* (fungal species) for antimicrobial activities of plant extracts[11,18].

Preparation of Sterile Paper Discs:

Using an ordinary office two-hole puncher, paper disks with approximate diameter of 6.3 mm. were punched out one by one from a sheet of blotting paper, the disks were placed in boiling test tubes then autoclaved for 15 minutes at 15 lbs. pressure and allowed to cool.

Medium for Bacterial Species:

Nutrient Broth/Nutrient Agar Medium (NBM/NAM) composition:

Peptone-5gm
Beef extract-3gm
Agar-5 gm
Distilled water-1000 ml
P^H - 7

Medium for Fungal Species:

Potato Dextrose Agar Medium (PDAM) ingredients:

Potato-20 gm
Dextrose-20 gm
Agar-20 gm
Streptomysin-30 gm
Distilled water -1000 ml
P^H - 7

Preparation of Test Plates for Antimicrobial Screening Tests

The Nutrient Agar (NA) and Potato Dextrose Agar (PDA) test plates (Petri dishes) were prepared by pouring about 15 ml of the medium. These test plates were placed under aseptic conditions at 4^0 C for 24 hours to control sterility. After solidifying the media (NA &PDA). The inoculums (bacteria 24 hrs and fungi 48 hrs.) Stock cultures were uniformly spread on their respective test plates. The filter paper discs were prepared in ethanol, methanol (M) and acetone (A) extracts as taken for control[11,24].

The filter paper discs are carefully placed the spreaded culture test plates are incubated at appropriate temperature for bacteria at 37 ^{0}C for 24 hrs. and fungi at 30 ^{0}C for 48 hrs. After the incubation period the test plates are examined for inhibitory zones are recorded. All determinants were made at least in triplicate for each of the test organisms in different extracts was also recorded [26,28].

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RESULTS AND DISCUSSIONS

A total no. of 92 ethanol, methanol, Hot water and acetone solvent extracts belongs to the 7 plant species of individual, combinations (Lf.) and multiple were subjected for antifungal and antimicrobial screening, in that all the 80 samples are exhibited positive inhibition zone activity[2,18]. The observations are recorded and they have been categorized into high or maximum zone (cognizable inhibitory zone) (i.e.5-10 mm inhibition zone) in 28 samples of (Ethanol/Methanol/Acetone extracts), moderate inhibition zone of expression in 19 samples of (E/M/A extracts) (i.e. 5-10 mm inhibition zone) and minimal inhibition zone of expression in 75 samples of (i.e. < 15 mm inhibition zone).

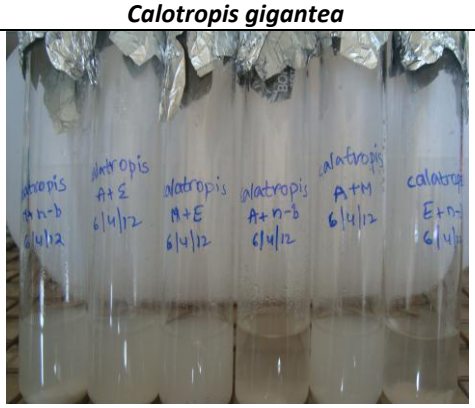
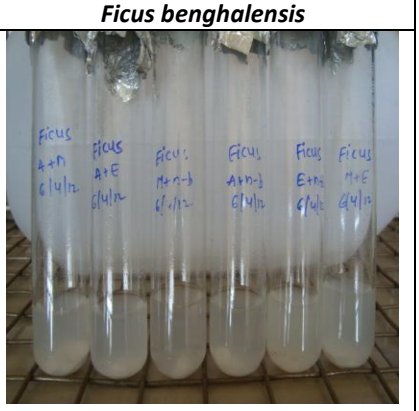
The inhibitory activity i.e. 5to 25 mm zone of expression for plant samples (Ethanol extracts) for individual in 7 samples, in combination 3samples i.e. in total= 21samples. 25 to 30mm zone of expression for plant samples (Methanol extracts) for individual in 7 samples, in combination 2 samples i.e. in total=14 samples and 25 to 30mm zone of expression for plant samples (Acetone extracts) for individual in 7 samples and combination 2samples i.e. in total =14 samples. (Table:1,2,3,4,5,6,7,8, 9,10,11,12,13,14&15) Ethanol extracts are comparatively effective more than those of methanol, acetone, hot water and n-butanol.

CONCLUSIONS

The present work will focus on the importance of latex and their antifungal and antibacterial activity. The latex was collected from different plants to test their antifungal and antibacterial activity.

The Antifungal activity and the anti bacterial activity were tested against 7 plant latex samples. A total number of 92 plant extracts (latex samples) were prepared as an individual of 28 ethanol, methanol, acetone and hot water extracts belongs to the 7 individual plant species and another set of 6 plant extracts (latex samples) were prepared in combination of 36 ethanol, methanol, acetone & n-butanol. 6 plant extracts (latex samples) were prepared in multiple combinations of 24 ethanol, methanol, acetone, n-butanol. All these plant extracts (latex samples) were subjected against their antibacterial and anti fungal screening analysis, out of these plant extracts (individual, combination & multiple) 5 plant extracts have been expressed the cognizable zone of expression i.e. 5 mm > 25 mm inhibition (Table:1,2,3,4,5,6,7,8,9,10,11,12,13,14,&15). Therefore these combinations were suggested for further analysis of latex for their antimicrobial activity.

COMBINATION/S – EXTRACTS

Plumeria alba	*Calotropis gigantea*	*Ficus benghalensis*
		
Acetone+Ethanol (A+E) Acetone+ Methanol(A+M) Acetone+n-butanol (A+n-bt) Methanol+Ethanol (M+E) Methanol+ n-butanol(M+n-bt) Ethanol + n-butanol (E+ n-bt)	Acetone + Ethanol (A+E) Acetone + Methanol (A+M) Acetone + n-butanol (A+ n-bt) Methanol+Ethanol (M+E) Methanol+ n-butanol (M+ n-bt) Ethanol + n-butanol (E+ n-bt)	Acetone + Ethanol (A+E) Acetone + Methanol (A+M) Acetone + n-butanol (A+ n-bt) Methanol+Ethanol (M+E) Methanol+n-butanol (M+n-bt) Ethanol + n-butanol (E+ n-bt)

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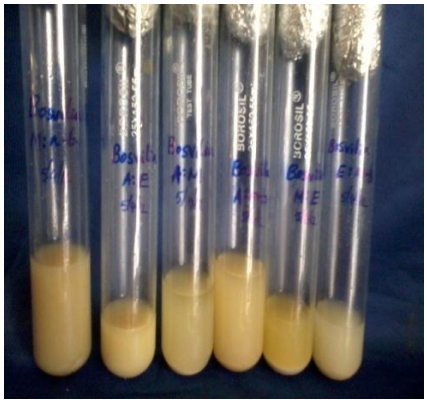
Ficus religiosa



Acetone + Ethanol (A+E)
Acetone + Methanol (A+M)
Acetone + n-butanol (A+ n-bt)
Methanol+Ethanol (M+E)
Methanol+ n-butanol (M+ n-bt)
Ethanol + n-butanol (E+ n-bt)

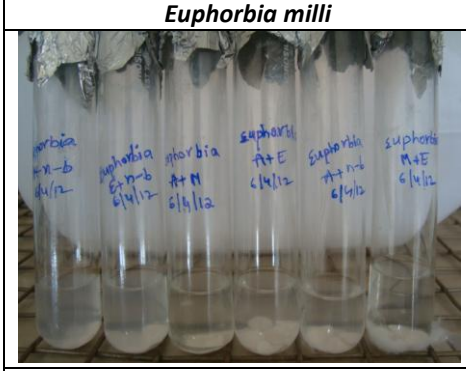
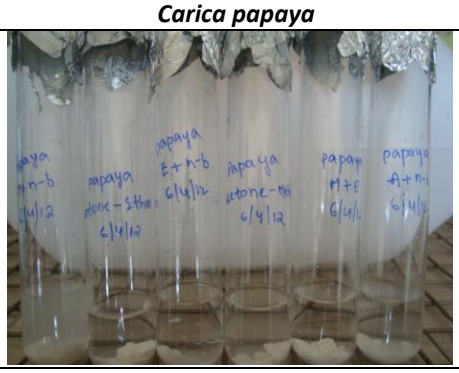
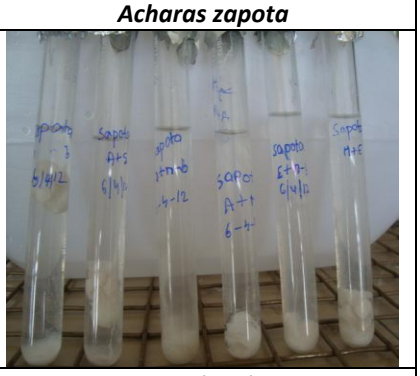
Euphorbia milli	***Carica papaya***	***Acharas zapota***
		
Acetone+Ethanol (A+E) Acetone+ Methanol(A+M) Acetone+n-butanol (A+n-bt) Methanol+Ethanol (M+E) Methanol+ n-butanol(M+n-bt) Ethanol + n-butanol (E+ n-bt)	Acetone+Ethanol (A+E) Acetone+ Methanol(A+M) Acetone+n-butanol (A+n-bt) Methanol+Ethanol (M+E) Methanol+ n-butanol(M+n-bt) Ethanol + n-butanol (E+ n-bt)	Acetone+Ethanol (A+E) Acetone+ Methanol(A+M) Acetone+n-butanol (A+n-bt) Methanol+Ethanol (M+E) Methanol+ n-butanol(M+n-bt) Ethanol + n-butanol (E+ n-bt)

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TABLE:1 Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Bacillus cereus.*

S.No.	Name of the Plant	Name of the organism	5 to10 mm				10 to12 mm				12 to15 mm				>15 mm(15-20mm)			
			A	E	M	HW	A	E	M	H W	A	E	M	H W	A	E	M	H W
1.	*Euphorbia mili*	*Bacillus cereus*	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*Bacillus cereus*	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+
3.	*Ficus bengalensis*	*Bacillus cereus*	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*Bacillus cereus*	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*Bacillus cereus*	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
6.	*Caltropis gigantea*	*Bacillus cereus*	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	+
7.	*Carica papaya*	*Bacillus cereus*	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+

A: Acetone, E: Ethanol, M: Methanol, H.W.: Hot water

TABLE:2 Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *E.coli*

S.No.	Name of the Plant	Name of the organism	5 to10 mm				10 to12 mm				12 to15 mm				>15 mm (15-20mm)			
			A	E	M	H W	A	E	M	H W	A	E	M	H W	A	E	M	H W
1.	*Euphorbia mili*	*E.coli*	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*E.coli*	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus benghalensis*	*E.coli*	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-
4.	*Ficus religiosa*	*E.coli*	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-
5.	*Plumeria alba*	*E.coli*	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
6.	*Caltropis gigantea*	*E.coli*	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-
7.	*Carica papaya*	*E.coli*	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+

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TABLE: 3-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *A.niger.*

S.No.	Name of the Plant	Name of the organism	5 to10 mm				10 to12 mm				12 to15 mm				>15 mm(15-20mm)			
			A	E	M	H W	A	E	M	H W	A	E	M	H W	A	E	M	H W
1.	*Euphorbia mili*	*Aspergillus niger*	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
2.	*Acharas zapota*	*Aspergillus niger*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
3.	*Ficus benghalensis*	*Aspergillus niger*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*Aspergillus niger*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*Aspergillus niger*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*Aspergillus niger*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
7.	*Carica papaya*	*Aspergillus niger*	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-

TABLE: 4-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Cercospora pongamiae (C. pongamiae)*

S.No.	Name of the Plant	Name of the organism	5 to10 mm				10 to12 mm				12 to15 mm				>15 mm (15-20mm)			
			A	E	M	H W	A	E	M	H W	A	E	M	H W	A	E	M	H W
1.	*Euphorbia mili*	*C. pongamiae*	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*C. pongamiae*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus benghalensis*	*C. pongamiae*	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-
4.	*Ficus religiosa*	*C. pongamiae*	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*C. pongamiae*	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*C. pongamiae*	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-
7	*Carica papaya*	*C. pongamiae*	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-

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TABLE:5-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Phytophthora infestans. (P. infestans)*

S.No.	Name of the Plant	Name of the organism	5 to10 mm				10 to12 mm				12 to15 mm				>15 mm (15-20mm)			
			A	E	M	H W	A	E	M	H W	A	E	M	H W	A	E	M	H W
1.	*Euphorbia mili*	*P. infestans*	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*P. infestans*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*P. infestans*	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*P. infestans*	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*P. infestans*	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-
6.	*Caltropis gigantea*	*P. infestans*	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
7.	*Carica papaya*	*P. infestans*	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-

COMBINATION/S-EXTRACTS:

Acetone + Ethanol; Ethanol + n-butanol; Acetone + Methanol; Acetone + n-butanol; Methanol + Ethanol; Methanol + n-butanol

TABLE: 6-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Bacillus cereus.(B.cereus)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm						10 to 15mm						15 to 20mm						>20mm (20-25mm)					
			A + E	E + n-b	A + M	A + n-b	M + E	M + n-b	A + E	E + n-b	A + M	A + n-b	M + E	M + n-b	A + E	E + n-b	A + M	A + n-b	M + E	M + n-b	A + E	E + n-b	A + M	A + n-b	M + E	M + n-b
1.	*Euphorbia mili*	*B. cereus*	-	-	-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*B. cereus*	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*B. cereus*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Carica papaya*	*B. cereus*	-	-	-	-	+	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*B. cereus*	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*B. cereus*	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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TABLE:7-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *E.coli*

S.No.	Name of the Plant	Name of the organism	5 to 10mm						10 to 15mm						15 to 20mm						>20mm (20-25mm)					
			A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b
1.	*Euphorbia mili*	*E.coli*	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*E.coli*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*E.coli*	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Carica papaya*	*E.coli*	-	-	+	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*E.coli*	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*E.coli*	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE: 8-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Phytophthora infestans (P. infestans)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm						10 to 15mm						15 to 20mm						>20mm (20-25mm)					
			A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b
1.	*Euphorbia mili*	*P. infestans*	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*P. infestans*	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*P. infestans*	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-
4.	*Carica papaya*	*P. infestans*	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*P. infestans*	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*P. infestans*	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+	-	-

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TABLE: 9-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Cercospora pongamiae (C. pongamiae)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm						10 to 15mm						15 to 20mm						>20mm (20-25mm)					
			A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-B	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b
1	*Euphorbia mili*	*C. pongamiae*	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	*Acharas zapota*	*C. pongamiae*	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3	*Ficus bengalensis*	*C. pongamiae*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-
4	*Carica papaya*	*C. pongamiae*	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5	*Plumeria alba*	*C. pongamiae*	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	*Caltropis gigantea*	*C. pongamiae*	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

TABLE:10- Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Aspergillus niger (A. niger).*

S.No.	Name of the Plant	Name of the organism	5 to 10mm						10 to 15mm						15 to 20mm						>20mm (20-25mm)					
			A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-B	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b	A+E	E+n-b	A+M	A+n-b	M+E	M+n-b
1.	*Euphorbia mili*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*A. niger*	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Carica papaya*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*A. niger*	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

MULTIPLE COMBINATION/S :

1) ACETONE+N-BUTANOL +ETHANOL (A+n-b+E)
2) ETHANOL+ N-BUTANOL+METHANOL (E+n-b+M)
3) METHANOL +ACETONE+ N-BUTANOL (M+A+n-b)
4) ACETONE+ ETHANOL+ METHANOL (A+E+M)

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TABLE: 11-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Bacillus cereus (B. cereus)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm				10 to 15mm				15 to 20mm				>20mm (20-25mm)			
			A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M
1.	*Euphorbia mili*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*B. cereus*	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*B. cereus*	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
7.	*Carica papaya*	*B. cereus*	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE:12- Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *E.coli*

S.No.	Name of the Plant	Name of the organism	5 to 10mm				10 to 15mm				15 to 20mm				>20mm (20-25mm)			
			A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M
1.	*Euphorbia mili*	*E.coli*	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+
2.	*Acharas zapota*	*E.coli*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*E.coli*	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*E.coli*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*E.coli*	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*E.coli*	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
7	*Carica papaya*	*E.coli*	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-

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TABLE:13-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Phytophthora infestans (P. infestans)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm				10 to 15mm				15 to 20mm				>20mm (20-25mm)			
			A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M
1.	*Euphorbia mili*	***P. infestans***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	***P. infestans***	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	***P. infestans***	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
4.	*Ficus religiosa*	***P. infestans***	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	***P. infestans***	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	***P. infestans***	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7.	*Carica papaya*	***P. infestans***	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

TABLE: 14-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Cercospora pongamiae (C. pongamiae)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm				10 to 15mm				15 to 20mm				>20mm (20-25mm)			
			A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M
1.	*Euphorbia mili*	*C. pongamiae*	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*C. pongamiae*	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*C. pongamiae*	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*C. pongamiae*	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*C. pongamiae*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*C. pongamiae*	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
7	*Carica papaya*	*C. pongamiae*	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

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TABLE:15-Showing the antimicrobial activity of (Zone of inhibition 5mm to >/=20mm) latex subjected to *Aspergillus niger (A.niger)*

S.No.	Name of the Plant	Name of the organism	5 to 10mm				10 to 15mm				15 to 20mm				>20mm (20-25mm)			
			A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M	A + n-b + E	E + n-b + M	M + A + n-b	A + E + M
1.	*Euphorbia mili*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*Acharas zapota*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	*Ficus bengalensis*	*A. niger*	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
4.	*Ficus religiosa*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	*Plumeria alba*	*A. niger*	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
6.	*Caltropis gigantea*	*A. niger*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7.	*Carica papaya*	*A. niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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