

Antimicrobial Activity of Fruit Latexes from Ten Laticiferous Plants

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Abstract

In the present investigation antibacterial activity of latexes from ten Indian plant species *Spondias dulcis* (Amra), *Diospyros melanoxylon* (Tendu), *Terminalia bellirica* (Wahera), *Ficus glomerata* (Gular), *Phyllanthus emblica* (Awla), *Thevetia neriifolia* (Kaner), *Carica papaya* (Papita), *Calotropis procera* (Ak), *Ficus benghalensis* (Bargad), *Artocarpus heterophyllus* (Katha) collected from Gorakhpur, North India were determined in various *in vitro* systems. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values were determined in growth inhibitory bioassays by using different increasing concentrations of various latex extracts. Latex samples were diluted by using serial micro dilution method up to 10^{-10} method with Luria broth culture medium. These values were obtained significantly 2 - 3 times lower than that of broad spectrum antibiotic drugs. Besides this, inhibition zone diameters were measured in agar disc diffusion assay. A known volume *i.e.* 0.1 - 20 $\mu\text{g}/\mu\text{l}$ of each latex were coated on separate sterile filter paper discs (Whatman No. 1) measuring 6 mm in size. Latex fractions registered significantly higher growth inhibition than that of broad spectrum antimicrobial drugs. Present study indicates the potential use of shows that both latex and its components and latex as are valuable source of medicinal products/active principles that can be used for treatment of life threatening infectious diseases. Because of higher inhibitory and cidal potential obtained in latexes than the synthetic drugs these that could lead to become efficient phytomedicines mainly to have and develop as complete drug formulations against to control infectious microbes.

Keywords

Plant Latex, *Carica papaya*, *Calotropis procera*, *Ficus benghalensis*, *Artocarpus heterophyllus*, Growth Inhibition, Antimicrobial Susceptibility

1. Introduction

Latex is milky fluid secreted by the ducts of laticiferous tissue found in roots, stems, leaves and fruits of flower-

ing plants [1]. It is a mixture of many pharmaceutically active substances which showed very high anti-bacterial [2], antiviral [3], antifungal [4] anti-protozoan activity. Latex from several plant species such as *A. ochroleuca* [2] *Calotropis procera*, *Plumeria rubra*, *Carica candamarcensis*, *Euphorbia tirucalli* (EtLP) [5], *Erythrina variegata* [6], *Jatropha curcas*, *Hancornia speciosa* Gomes (Apocynaceae) [7], *C. procera* [8], and *Euphorbia lathyris* [9] have shown very high antimicrobial activity. The leaves of *J. curcas* contain apigenin, vitexin and isovitexin, which along with other factors showed strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [10]. *Hevea brasiliensis* showed antifungal activity against *Trichosporon cutaneum* and *Cryptococcus neoformans* [5]. It also exhibit good activity against multiple drug resistant *Staphylococcus aureus* (NCTC 11994) and *Salmonella typhimurium* (ATCC 1255) and *Candida albicans* (ATCC 10231) [11]. The latex of fig fruit (*Ficus carica*) is used in traditional medicine for the treatment of skin infections. Its hexanic and hexane-ethyl acetate (v/v) extracts inhibit virus replication and work against herpes simplex type 1 (HSV-1), echovirus type 11 (ECV-11) and adenovirus (ADV) infections [12]. Latex from *Phyllanthus* sp. showed antiviral effects [13].

Plant latex is a natural source of antiprotozoal drugs [14] [15] which show schizonticidal activity [16]. Poly-o-acylated jatrophone diterpenes found in latex of *Pedilanthus tithymaloides* showed antiplasmodial activity [17]. Similarly *Xylopiya aromatica*, *Casearia sylvestris*, *Cupania vernalis* and *Aspidosperma macrocarpon* [18] *Casearia sylvestris* var. *lingua* latex showed anti-protozoan activity against *Trypanosoma cruzi* [19]. *C. scoparioides* latex shows antimalarial, leishmanicidal and trypanocidal activity *in vitro* [20]. Latex from few plant species such as *Himatonthus sucouba* [21] showed Leishmanicidal activity while lectins isolated from *Synadenium carinatum* latex exhibit anti-protozoan activity against *Leishmania, amazonensis* promastigotes/amastigotes [22]. Moreover, *Croton lechleri* latex contains crofelemer which shows anti-secretory antidiarrheal activity [23].

In present time drug resistance in microbes is a very serious problem. With the time new chemical drugs have been discovered for control of these pathogens. But due to indiscriminate use of these synthetic drugs, most of the pathogens have developed resistance. Though in the beginning, these synthetic antibiotics have shown very good fighting potential against pathogens but later on these become ineffective because microbes have developed resistance to them. Besides, synthetic drugs generate wider side effects and heavy cross reactivity in the body tissues and cells. Therefore, for the treatment of infectious diseases, many complementary and alternative medicines are to be discovered for safe therapeutic purposes. In present time for effective control of microbial infections, plant products considered as best solutions because of their low side effects. Until the date, very few active components have been isolated from plants and still their antimicrobial susceptibility is yet to check. In spite of the fact lot of work has been done on antimicrobial plant product but no such work is reported on plant latex. Hence, in the present investigation antimicrobial susceptibility of plant latex was screened for effective control of infectious pathogens. Until the date, very few active components have been isolated from plants and still their antimicrobial susceptibility is yet to check. In spite of the fact, lot of work has been done on antimicrobial plant product but no such work is reported on plant latex. Hence, in the present investigation antimicrobial susceptibility of plant latex was screened for effective control of infectious pathogens. The main objective of the present study was to establish the antibacterial activity of fruit latex from four plant species *Spondias dulcis* (Amra), *Diospyros melanoxylon* (Tendu), *Terminalia bellirica* (Wahera), *Ficus glomerata* (Gular), *Phyllanthus emblica* (Awla), *Thevetia neriifolia* (Kaner), *Carica papaya* (Papita), *Calotropis procera* (Ak), *Ficus benghalensis* (Bargad), *Artocarpus heterophyllus* (Kathal) (Figure 1) pathogenic bacterial strains *i.e.* *Klebsiella pneumoniae*, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Micrococcus luteus* and *Streptococcus pneumoniae*.

2. Experimental

2.1. Plant Material

Fruit latexes were collected from unripe fresh fruits in a aseptic glass vessel by making sharp incisions at fixed time interval [24]. For draining maximum amount fruits were cut open from its top then slightly squeezed to collect the latex in sterile tube then stored at -20°C until further use. Fruit latexes from eight plant species *Spondias dulcis* (Amra), *Diospyros melanoxylon* (Tendu), *Terminalia bellirica* (Wahera), *Ficus glomerata* (Gular), *Phyllanthus emblica* (Awla), *Thevetia neriifolia* (Kaner), *Carica papaya* (Papita), *Calotropis procera* (Ak), *Ficus benghalensis* (Bargad), *Artocarpus heterophyllus* (Kathal) (Figure 1) were used for various anti-microbial bioassays.



1



2



3



4



5



6



7a



7b



Figure 1. Showing various latex bearing fruits from plant species i.e. 1. *Spondias dulcis* (Amra), 2. *Diospyros melanoxylon* (Tendu), 3. *Terminalia bellirica* (Wahera), 4. *Ficus glomerata* (Gular), 5. *Phyllanthus emblica* (Awla), 6. *Thevetia nerifolia* (Kaner), 7(a & b) *Carica papaya* (Papita), 8. *Calotropis procera* (Ak), 9. *Ficus benghalensis* (Bargad), 10 (a & b) *Artocarpus heterophyllus* (Kathal).

2.2. Isolation and Extraction of Plant Latex

Collected plant latex was lyophilized and powdered in vacuum in cold. It was extracted with different solvents. For better fractionation active fractions from the latex were portioned between different solvents on the basis of their polarity from low to high and non polar. Mostly portioning was done between hexane and aqueous methanol, petroleum ether and chloroform. Further a portion of dried latex was extracted with distilled water, 1.5% acetic acid, 1.5% Sodium bicarbonate and 1.5% sulfuric acid and diethyl ether to separate various fractions by following the method of Steven McCay and Paul Mahlberg, 1973 [25]. For testing antimicrobial activity a known quantity of vacuum dried plant latex was added to fresh solvents. Latex samples were stored in cold at 4°C.

2.3. Maintenance of Bacterial Culture

Cultures of seven pathogenic bacterial strains each of *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Lactobacillus acidophilus* (ATCC 53103), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae*, (ATCC 15380) and *Streptococcus pneumoniae* (ATCC 12755) was maintained in the laboratory in Luria Broth (2% w/v) regularly for five days at 37°C before use in experiments. For experiments, a portion (100 µl) of the overnight bacterial culture was mixed in the tests and control for inoculation. For activity anti-microbial testing bacterial cultures were sub cultured after every 8th day in solid agar plates and stored at 4°C.

2.4. Determination of Antimicrobial Activity: MIC and MBC Values

For determination of antimicrobial activity bacterial growth inhibition was accessed in presence of different increasing concentrations of plant latex fractions. For this purpose, latex samples were diluted by using serial mi-

cro dilution method with Luria Broth culture medium. In tests latex was added to fresh suspension after making the serial dilutions up to 10^{-10} . Each sample of latex was assayed in triplicate. Before conducting experiments all the conditions for *in vitro* anti-microbial activity were standardized to determine MIC and MBC values according to Karamon *et al.*, 2003 [26]. The MIC values were determined in culture flask after 24 h of incubation at 37°C. The turbidity in the culture flasks was considered as visible growth of microorganisms. Further, it was standardized in terms of absorbance at 600 nm in a visible spectrophotometer. For determination of minimum bactericidal concentration (MBC) growth inhibitory assays were also performed. For this purpose, inoculum size was adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml) in sterile agar plates. The incubation of test and control cultures was performed at 37°C for 24 h. For comparison, both negative and positive controls were set and bacterial colony number was counted in all test and control Petri plates. The least concentration at which no visible growth obtained in agar plates was considered as MBC value. For evaluation of inhibition two parallel controls were set for each test sample.

2.5. Determination of Inhibition Zone Diameter

Antimicrobial activity of latex was evaluated by agar disc diffusion method and inhibition zone diameters were measured according to Bauer *et al.*, 1966 [27] in presence of each latex sample separately. Agar-agar was used as media for the test microorganisms. For antimicrobial activity testing plant latex samples were diluted by adding equal volume of solvent and preceded further two to obtain two fold serial micro-dilution. From this, a known volume *i.e.* 2 - 32 µl of each latex coated on sterile filter paper discs (Whatman No. 1) measuring 6 mm in size. These latex impregnated discs were dried under laminar flow cabinet. Before experiment inoculum size was determined and adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml) in sterile agar plates. Bacterial inoculum was evenly distributed on to the surface of agar plates by using sterile rubber pad spreader. Now latex coated discs were positioned on the inoculated agar surface. Each latex sample was assayed in triplicate. Sterile distilled water was used as negative control while different antibiotics *i.e.* tetracycline, ampicillin and ciprofloxacin were used as standard (positive control) to compare the bacterial growth. All treated and untreated plates were incubated for 24 h at 37°C. Inhibition zone diameter surrounding the filter paper discs were measured in each test and control Petri plate.

3. Results

3.1. Determination of MIC and MBC Values

The antimicrobial activity of solvent and aqueous extracts from fruit latexes of eight plant species were tested against seven pathological strains of bacteria. The MIC values obtained *in vitro* suspension culture of different plant extracts are shown in **Table 1**. The MIC value of all the fruit latex extracts was found to be very low. The MIC value for *Spondias dulcis* (Amra), hexanoic extract was obtained lowest against *S. pneumoniae* (0.12 µg/mL), while methanolic extract was found more susceptible to *Klibesella pneumoniae* as the MIC value was lowest *i.e.* 0.28 µg/mL. Its petroleum extract and chloroform extracts have shown higher activity against *Klibesella pneumoniae* as MIC values obtained were 0.64 and 0.40 µg/mL. Its water extract has shown higher activity against *E. coli*, *B. cereus* and *Lactobacillus acidophilus* as the MIC value obtained was 0.72 µg/mL (**Table 1**). *Diospyros melanoxylon* (Tendu), methanolic extract has shown higher MIC value 0.070 µg/mL against *K. pneumoniae*, while hexanoic extract *i.e.* 0.48 µg/mL against *S. pneumoniae*. Its water extract has shown MIC value 0.72 µg/mL against *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* (**Table 1**). *Terminalia bellirica* (Wahera), methanolic extract has shown higher MIC value against *E. coli* and *M. luteus* 0.14, and 0.24 µg/mL while water extract has shown lowest MIC value 0.72 *E. coli* and *Lactobacillus acidophilus* (**Table 1**). *Ficus glomerata* (Gular) methanolic extract has shown higher MIC value against *Klebsiella pneumoniae*, *M luteus* 0.14 µg/mL and 0.24 µg/mL against *Lactobacillus acidophilus* (**Table 1**). *Phyllanthus emblica* (Awla) methanolic extract has shown higher MIC value against *Klebsiella pneumoniae* and *Streptococcus pneumoniae* 0.14 µg/mL (**Table 1**). Its water extract has shown lowest MIC against 0.36 0.14 µg/mL against *Staphylococcus aureus* and *Lactobacillus acidophilus*. Methanolic extract of *Thevetia neriifolia* (Kaner), has shown lower MIC value 0.14 µg/mL and 0.24 µg/mL against *E. coli* and *S. aureus*. Its water extract was found active against three bacterial strains as the MIC values obtained are very low *E. coli*, *B. cereus* *Lactobacillus acidophilus* 0.72 µg/mL. *Calotropis procera* has shown very high antimicrobial susceptibility as the MIC value obtained was least in all cases.

Table 1. Determination of MIC and MBC ($\mu\text{g/ml}$) values of aqueous and solvent extracts of different plant latexes against certain bacterial strains.

Fractions	Conc. range in ($\mu\text{g/ml}$)	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>M. luteus</i>	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>L. acidophilus</i>
<i>Spondias dulcis</i> (Amra)								
Hexanoic	0.030 - 30.72	0.96 (2.76)	1.92 (3.84)	0.48 (0.96)	0.12 (0.48)	0.96 (1.92)	0.24 (0.96)	0.12 (0.24)
Methanolic	0.035 - 35.84	0.28 (1.12)	0.56 (1.12)	0.24 (0.96)	0.24 (0.48)	0.48 (0.96)	1.92 (7.68)	0.96 (1.92)
Pet. Ether	0.040 - 40.96	0.64 (2.56)	0.64 (1.28)	2.56 (5.12)	0.64 (1.28)	1.28 (5.12)	1.28 (5.12)	2.56 (5.12)
Chloroform	0.025 - 51.20	0.40 (1.60)	1.60 (3.20)	1.60 (3.20)	0.80 (1.60)	0.80 (1.60)	1.60 (3.20)	3.20 (6.40)
Water	0.045 - 46.08	1.44 (2.88)	0.72 (2.88)	1.44 (2.88)	1.44 (2.88)	2.88 (5.76)	0.72 (2.88)	0.72 (2.88)
<i>Diospyros melanoxylon</i> (Tendu)								
Hexanoic	0.030 - 30.72	0.96 (1.92)	1.92 (3.84)	0.96 (1.92)	0.48 (0.96)	1.92 (3.84)	1.92 (3.84)	0.96 (3.84)
Methanolic	0.035 - 35.84	0.070 (0.28)	0.14 (0.56)	0.28 (0.56)	0.56 (2.24)	0.28 (0.56)	0.28 (1.12)	0.056 (1.12)
Pet. Ether	0.040 - 40.96	0.64 (2.56)	1.28 (2.56)	1.28 (2.56)	2.56 (5.12)	0.80 (0.16)	0.28 (1.12)	0.32 (0.64)
Chloroform	0.025 - 51.20	0.20 (0.80)	3.20 (6.40)	1.60 (3.20)	0.40 (1.60)	1.60 (3.20)	0.80 (1.60)	0.80 (1.60)
Water	0.045 - 46.08	0.72 (2.88)	1.44 (2.88)	2.88 (5.76)	0.72 (2.88)	0.72 (2.88)	2.88 (5.76)	1.44 (2.88)
<i>Terminalia bellirica</i> (Wahera)								
Hexanoic	0.030 - 30.72	0.48 (0.96)	0.96 (1.92)	1.92 (3.84)	0.48 (0.96)	3.84 (7.68)	3.84 (7.68)	0.48 (1.92)
Methanolic	0.035 - 35.84	0.56 (1.12)	0.14 (0.24)	0.24 (0.48)	0.24 (1.12)	0.56 (1.12)	1.12 (2.24)	0.56 (1.12)
Pet. Ether	0.040 - 40.96	0.64 (2.56)	0.64 (2.56)	1.28 (2.56)	1.28 (5.12)	1.28 (2.56)	0.64 (2.56)	0.64 (1.28)
Chloroform	0.025 - 51.20	0.40 (1.60)	1.60 (3.20)	0.80 (1.60)	0.40 (1.60)	0.40 (1.60)	0.80 (1.60)	0.40 (1.60)
Water	0.045 - 46.08	1.44 (2.88)	0.72 (2.88)	1.44 (2.88)	1.44 (2.88)	2.88 (5.76)	1.44 (2.88)	0.72 (2.88)
<i>Ficus glomerata</i> (Gular)								
Hexanoic	0.030 - 30.72	0.96 (1.92)	1.92 (3.84)	0.96 (3.84)	0.48 (1.92)	0.48 (1.92)	1.92 (3.84)	0.48 (0.96)
Methanolic	0.035 - 35.84	0.14 (0.28)	0.24 (0.48)	0.14 (0.28)	0.56 (1.12)	0.56 (1.12)	0.56 (1.12)	0.28 (0.56)
Pet. Ether	0.040 - 40.96	1.28 (2.56)	0.64 (2.56)	1.28 (2.56)	2.56 (5.12)	1.28 (2.56)	0.64 (1.28)	0.64 (1.28)
Chloroform	0.025 - 51.20	1.60 (3.20)	0.80 (1.60)	0.40 (1.60)	1.60 (3.20)	1.60 (3.20)	0.80 (1.60)	0.40 (1.60)
Water	0.045 - 46.08	0.36 (1.44)	1.44 (2.88)	2.88 (5.76)	0.72 (2.88)	0.72 (2.88)	1.44 (2.88)	1.44 (2.88)
<i>Phyllanthus emblica</i> (Awla)								
Hexanoic	0.030 - 30.72	1.92 (3.84)	1.92 (7.68)	3.84 (6.78)	1.92 (3.28)	0.48 (0.96)	0.48 (0.96)	0.96 (1.92)
Methanolic	0.035 - 35.84	0.14 (0.56)	0.28 (0.56)	0.14 (1.12)	0.14 (0.56)	1.12 (2.24)	1.12 (2.24)	0.56 (1.12)
Pet. Ether	0.040 - 40.96	0.64 (1.28)	1.28 (2.56)	0.64 (2.56)	1.28 (2.56)	2.56 (5.12)	0.64 (1.28)	0.64 (2.56)
Chloroform	0.025 - 51.20	0.80 (1.60)	0.40 (1.60)	1.60 (3.20)	0.40 (1.60)	0.80 (1.60)	0.80 (1.60)	0.40 (1.60)
Water	0.045 - 46.08	0.72 (2.88)	0.72 (2.88)	0.72 (2.88)	0.36 (1.44)	0.36 (1.44)	0.72 (2.88)	0.36 (1.44)
<i>Thevetia neriifolia</i> (Kaner)								
Hexanoic	0.030 - 30.72	0.96 (1.92)	0.24 (0.96)	0.96 (1.92)	0.48 (1.92)	0.96 (4.84)	1.92 (3.84)	0.96 (1.92)
Methanolic	0.035 - 35.84	0.28 (1.12)	0.14 (1.12)	0.28 (2.24)	0.56 (1.12)	0.14 (0.42)	0.28 (2.24)	0.28 (2.24)
Pet. Ether	0.040 - 40.96	0.64 (5.12)	0.32 (0.64)	0.64 (2.56)	0.64 (2.56)	2.56 (5.12)	2.56 (5.12)	0.64 (0.128)
Chloroform	0.025 - 51.20	0.40 (1.60)	1.60 (3.20)	0.80 (1.60)	0.40 (1.60)	0.80 (1.60)	1.60 (3.20)	0.40 (1.60)
Water	0.045 - 46.08	1.44 (2.88)	0.72 (2.88)	1.44 (2.88)	1.44 (2.88)	1.44 (2.88)	0.72 (2.88)	0.72 (1.44)

Continued

<i>Carica papaya</i> (Papita)								
Hexanoic	0.030 - 30.72	0.24 (0.48)	0.48 (0.96)	0.48 (1.92)	2.92 (3.84)	0.96 (1.92)	0.48 (1.92)	0.96 (1.92)
Methanolic	0.035 - 35.84	0.28 (0.56)	0.14 (0.56)	0.28 (0.56)	0.56 (1.12)	0.28 (0.56)	0.28 (0.56)	0.14 (0.56)
Pet. Ether	0.040 - 40.96	0.32 (0.64)	0.64 (1.28)	0.64 (1.28)	1.28 (2.56)	1.28 (2.56)	2.56 (1.28)	0.64 (1.28)
Chloroform	0.025 - 51.20	0.80 (1.60)	0.40 (0.80)	0.80 (1.60)	0.80 (1.60)	0.20 (0.40)	0.80 (1.60)	0.40 (0.80)
Water	0.045 - 46.08	0.36 (1.44)	0.72 (1.44)	1.44 (2.88)	0.44 (2.88)	0.72 (2.88)	0.36 (0.72)	0.72 (1.44)
<i>Calotropis procera</i> (Ak)								
Hexanoic	0.030 - 30.72	0.48 (0.96)	0.96 (1.92)	0.48 (1.92)	1.92 (3.84)	0.48 (1.92)	0.96 (1.92)	0.48 (1.92)
Methanolic	0.035 - 35.84	0.14 (0.56)	0.28 (0.56)	0.56 (1.12)	0.28 (1.12)	0.14 (0.24)	0.28 (0.56)	0.56 (1.12)
Pet. Ether	0.040 - 40.96	0.64 (1.28)	0.32 (0.64)	0.32 (0.64)	1.28 (2.56)	2.56 (5.12)	2.56 (1.24)	0.64 (1.28)
Chloroform	0.025 - 51.20	0.40 (1.60)	0.80 (1.60)	0.40 (1.60)	0.40 (1.60)	0.80 (1.60)	0.80 (1.60)	0.80 (1.60)
Water	0.045 - 46.08	0.72 (1.44)	0.36 (1.44)	0.72 (2.88)	1.44 (2.88)	1.44 (2.88)	0.72 (1.44)	0.36 (0.72)
<i>Ficus benghalensis</i> (Bargad)								
Hexanoic	0.030 - 30.72	0.24 (0.48)	0.48 (1.92)	0.48 (1.92)	0.96 (3.84)	0.24 (1.92)	0.48 (1.92)	0.96 (1.92)
Methanolic	0.035 - 35.84	0.28 (0.56)	0.14 (0.56)	0.56 (1.12)	0.56 (1.12)	0.56 (0.24)	0.28 (0.56)	0.28 (0.56)
Pet. Ether	0.040 - 40.96	0.32 (1.28)	0.64 (1.28)	0.32 (0.64)	0.64 (1.28)	1.28 (2.56)	2.56 (1.24)	0.32 (0.64)
Chloroform	0.025 - 51.20	0.80 (1.60)	0.40 (0.80)	0.40 (1.60)	0.80 (1.60)	1.60 (3.20)	0.40 (0.80)	0.80 (1.60)
Water	0.045 - 46.08	0.72 (1.44)	0.72 (1.44)	1.44 (2.88)	1.44 (2.88)	0.72 (2.88)	0.36 (0.72)	0.36 (0.72)
<i>Artocarpus heterophyllus</i> (Katha)								
Hexanoic	0.030 - 30.72	0.96 (1.92)	0.48 (1.92)	1.92 (3.84)	1.92 (3.84)	0.96 (1.92)	1.92 (3.84)	0.96 (3.84)
Methanolic	0.035 - 35.84	0.28 (1.12)	0.28 (1.12)	0.56 (1.12)	0.56 (1.12)	1.12 (2.24)	0.14 (0.28)	1.12 (2.24)
Pet. Ether	0.040 - 40.96	0.64 (1.28)	0.64 (2.56)	0.64 (2.56)	0.32 (0.64)	0.32 (0.64)	0.64 (1.28)	1.12 (2.24)
Chloroform	0.025 - 51.20	0.80 (1.60)	0.40 (1.60)	0.80 (1.60)	1.60 (3.20)	0.80 (1.60)	1.60 (3.20)	1.60 (3.20)
Water	0.045 - 46.08	1.44 (2.88)	0.72 (1.44)	0.72 (1.44)	1.44 (2.88)	1.44 (2.88)	0.72 (1.44)	0.72 (1.44)
Antibiotics								
Tetracycline	0.15 - 38.4 µg/ml	4.8 (9.6)	0.8 (1.6)	1.60 (3.2)	1.60 (3.20)	4.8 (9.6)	1.60 (3.2)	0.80 (1.6)
Ampicillin	0.15 - 38.4 µg/ml	3.2 (6.4)	1.60 (3.20)	1.60 (3.2)	1.60 (3.2)	6.40 (12.8)	1.60 (3.2)	1.60 (3.2)
Ciprofloxacin	0.15 - 38.4 µg/ml	1.6 (3.20)	3.2 (6.4)	0.8 (1.6)	0.8 (1.6)	1.60 (3.20)	0.8 (1.6)	0.8 (1.6)

*Plant latexes were diluted by serial micro-dilution method with Luria broth culture media. **Antibiotic solutions were prepared by same method and concentration range was 0.15 - 38.4 µg/ml.

It was ranging from 0.14 - 0.56 µg/mL against different bacterial strains. Its water extract has shown good antimicrobial activity as the MIC value was ranging from 0.36 - 1.44 µg/mL (Table 1). Methanolic extract of *Artocarpus heterophyllus* has shown lowest MIC value 0.14 - 0.56 µg/mL against *Klebsiella pneumoniae*, *E. coli*, *M. luteus*, *Streptococcus pneumoniae* and *B. cereus* (Table 1). MIC values obtained in case of broad spectrum antibiotic drugs tetracycline, ampicillin and ciprofloxacin were higher than the latex samples. These were ranging from 0.8 - 6.40 µg/mL against different bacterial strains (Table 1).

Besides MIC values MBC values of latex extracts were also found lower than the broad spectrum antibiotics assayed for comparison. These are displayed in Table 1. *Spondias dulcis* (Amra) methanolic extract has shown lowest MBC value 0.48 µg/mL against *S. pneumoniae*. It was quite higher in water extract as 2.88 µg/mL in

each case. Similar MBC value was also obtained in *Diospyros melanoxyton* (Tendu), water extract. *Diospyros melanoxyton* methanolic extract has shown lower MBC value in *K. pneumoniae*, *E. coli*, *M. luteus* and *auerus* i.e. 0.56 µg/mL (Table 1). *Terminalia bellirica* (Wahera), methanolic extract has shown MBC value against *E. coli* and *M. luteus* 0.24 and 0.48 µg/mL while water extract has shown lowest MIC value 2.88 µg/mL against all bacterial strains (Table 1). *Ficus glomerata* (Gular) methanolic extract has shown lower MBC value 0.28 - 0.56 µg/mL against *Klebsiella pneumoniae*, *E. coli*, and *Lactobacillus acidophilus* (Table 1). Its water extract has shown MBC value 1.44 µg/mL *K pneumoniae*. *Phyllanthus emblica* (Awla) methanolic extract has shown MBC value 0.56 µg/mL against *Klebsiella pneumoniae*, *E. coli* and *Streptococcus pneumoniae* 0.14 µg/mL (Table 1). Its water extract has shown lowest MBC S value against *Streptococcus pneumoniae*, *Staphylococcus aureus* and *L. acidophilus* 1.44 µg/mL. Methanolic extract of *Thevetia nerifolia* (Kaner), *Calotropis procera* (Ak) and *Artocarpus heterophyllus* (katahal) showed lower MBC values ranging from 0.56 - 2.44 µg/mL while water extract from these plants have shown MBC S values between 0.72 - 2.88 µg/mL (Table 1). A higher MBC values were obtained in broad spectrum antibiotics which depict low susceptibility in of these drugs in comparison to latex extracts. The MBC values were noted in a range of 1.6 - 9.6 µg/mL (Table 1). This suggests that fruit latex extracts, possess strong bactericidal agents that can be, used as prevailing drugs for the control of bacterial growth *in vivo*.

3.2. Determination of Inhibition Zone Diameters

Besides determination of MIC and MBC values the effectiveness of all fruit latex extracts was confirmed by filter paper disc diffusion bioassay. After 24 hrs of *in vitro* Kirby Bauer test growth inhibition were measured in tests and controls. However, IZD obtained in presence of each latex extract was considered as final bactericidal marker. These are displayed in Table 2. On the basis of size of growth inhibition zone pathogens were divided in to three categories i.e. resistant (>7), intermediate (>12) and susceptible >18 mm respectively The zone of inhibition above 7 mm was considered as positive results. Methanolic extract of *Spondias dulcis* (Amra) methanolic extract has shown highest inhibition zone diameter 28.13 against *K. pneumoniae* followed by *B. cereus* 25.51, *M. luteus* 23.2, *L. acidophilus* 21.2. In its hexane extract highest inhibition zone diameter 20.36 mm against *S. aureus*, Petroleum ether extract against *M. luteus* 19.76, chloroform against *B. cereus* 16.10. Water extract has shown inhibition zone diameter 15.32 against *E. coli* (Table 2), Similarly methanolic extract of *D. melanoxyton* (Tendu) has shown inhibition zone diameter between 19.65 - 25.48 mm against all different pathogens subsequently. It has shown highest inhibition zone diameter 25.48 against *L. acidophilus* (Table 2). Its water extract has shown inhibition zone diameter 16.26 against *E. coli*. *Terminalia bellirica* (Wahera) methanolic extract has shown IZD 24.54 against *K. pneumoniae*. Its water extract has shown highest inhibition zone diameter 18.32 against *L. acidophilus*. *Ficus glomerata* (Gular) methanolic extract has shown highest inhibition zone diameter between 12.86 - 17.64 mm against all different bacterial strains *Phyllanthus emblica* (Amla). It has shown highest inhibition zone diameter against *M. luteus* 28.66 followed by *K. pneumoniae* 27.82 and 27.34 mm (Table 2). Its aqueous extract has shown IZD between 13.65 - 19.60 (Table 2). *Thevetia nerifolia* (kaner) hexanoic extract has shown highest IZD 24.36 mm against *L. acidophilus*. Methanolic extract has shown highest inhibition zone diameter against *L. acidophilus* 30.74 mm. Petroleum ether extract has shown 17.60 mm against *E. coli* (Table 2). Its water extract has shown highest inhibition zone diameter against *L. acidophilus* 18.46 mm. *Calotropis procera* solvent extract has shown inhibition zone diameter between 13.80 - 28.72 mm. Its water extract has shown highest inhibition zone diameter between 13.54 - 17.60. *Artocarpus heterophyllus* methanolic extract has shown highest inhibition zone diameter 26.43 mm against *B. cereus*. Its water extract has shown inhibition zone diameter against *E. coli* 21.24 mm. In all tests inhibition zone diameter was found higher in solvent extracts in comparison to water extract. Furthermore, all solvent and aqueous extracts of latexes from assayed from different plant species have shown significantly higher inhibition zone diameter than the broad spectrum antibiotics as these were noted 7.46 - 14.86 mm. From the experiments methanolic extract were found to be highly susceptible against all the bacterial strains. As their growth inhibition zone diameter was obtained between 17.6 - 31.63 mm in size (Table 2). This inhibition was observed as 4-µg concentration of each extract at 24 h in agar disc diffusion bioassay. It was also observed that solvent extracts have shown higher inhibition zone diameter in comparison to water extracts.

4. Discussion

In present study various antimicrobial susceptibility bioassays were conducted *in vitro* and most of the plant la-

Table 2. The quantitations of microbial activity of different aqueous and solvent extracts of plant latexes were measured by agar diffusion method. The effectiveness of different extracts is demonstrated by the size of the micro-organisms growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm.

Fractions	Conc. µg/disc	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>M. luteus</i>	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>L. acidophilus</i>
<i>Spondias dulcis</i> (Amra)								
Hexanoic	4.00	17.33	15.36	18.46	19.33	20.36	14.70	19.23
Methanolic	4.00	28.13	17.66	23.20	17.60	19.40	25.51	21.20
Pet. Ether	4.00	16.43	17.46	19.76	17.43	18.30	16.26	19.16
Chloroform	4.00	12.34	13.40	14.30	12.86	15.12	16.10	13.18
Water	4.00	14.30	15.32	13.38	14.64	12.80	14.50	16.80
<i>Diospyros melanoxylon</i> (Tendu)								
Hexanoic	4.00	21.31	17.42	13.40	15.60	13.40	14.11	13.22
Methanolic	4.00	24.35	21.71	20.21	19.65	24.33	23.37	25.48
Pet. Ether	4.00	14.66	17.37	18.63	17.72	19.56	20.20	15.47
Chloroform	4.00	13.67	14.50	17.24	13.23	14.50	19.60	13.80
Water	4.00	13.45	16.26	13.82	17.60	14.20	14.50	12.76
<i>Terminalia bellirica</i> (Wahera)								
Hexanoic	4.00	13.47	15.75	19.67	16.33	12.40	18.74	20.65
Methanolic	4.00	24.54	23.36	21.44	25.80	24.23	21.73	18.46
Pet. Ether	4.00	16.30	15.37	18.14	19.26	16.42	19.23	21.78
Chloroform	4.00	14.20	16.76	15.43	14.20	12.86	14.80	15.74
Water	4.00	13.62	18.80	13.20	13.40	14.25	13.72	18.32
<i>Ficus glomerata</i> (Gular)								
Hexanoic	4.00	21.53	19.53	17.33	25.26	21.33	20.46	25.30
Methanolic	4.00	25.43	23.53	20.56	29.60	22.76	29.53	31.63
Pet. Ether	4.00	21.16	17.57	23.24	19.30	23.57	17.86	15.84
Chloroform	4.00	13.26	16.74	14.80	14.24	15.63	12.80	14.78
Water	4.00	14.22	17.64	15.67	13.44	13.84	13.66	12.86
<i>Phyllanthus emblica</i> (Awla)								
Hexanoic	4.00	19.66	25.43	20.17	25.31	22.71	21.26	17.30
Methanolic	4.00	27.82	25.13	28.66	27.34	23.16	29.53	31.63
Pet. Ether	4.00	23.06	16.50	29.26	18.30	21.50	19.36	16.83
Chloroform	4.00	14.62	18.12	13.26	12.76	15.40	14.34	13.24
Water	4.00	13.65	19.6	13.56	13.82	13.20	14.10	14.64
<i>Thevetia nerifolia</i> (Kaner)								
Hexanoic	4.00	20.17	17.23	19.67	24.28	22.14	19.61	24.36
Methanolic	4.00	23.87	22.13	21.82	27.45	22.12	28.36	30.74
Pet. Ether	4.00	22.10	15.56	28.66	17.42	20.23	18.46	17.56
Chloroform	4.00	14.22	14.70	13.74	13.80	14.76	13.28	13.66
Water	4.00	13.60	15.42	14.24	13.88	12.80	14.70	18.46

Continued

<i>Carica papaya</i> (Papita)								
Hexanoic	4.00	17.24	19.33	15.65	21.26	23.46	19.67	25.23
Methanolic	4.00	23.67	21.18	20.64	29.34	25.34	23.20	20.24
Pet. Ether	4.00	17.54	16.21	19.33	18.66	21.27	18.56	19.30
Chloroform	4.00	15.46	16.80	14.10	15.72	13.77	14.22	15.33
Water	4.00	15.68	14.71	15.22	13023	15.24	13.38	16.26
<i>Calotropis procera</i> (Ak)								
Hexanoic	4.00	20.43	18.68	16.42	23.46	22.13	21.65	26.71
Methanolic	4.00	24.16	22.68	21.77	28.72	24.54	28.86	23.53
Pet. Ether	4.00	22.66	15.74	21.22	17.68	22.57	19.36	17.10
Chloroform	4.00	16.24	15.20	13.80	14.60	17.23	13.80	13.67
Water	4.00	17.60	14.22	13.54	14.46	16.30	13.68	14.56
<i>Ficus benghalensis</i> (Bargad)								
Hexanoic	4.00	21.20	17.24	15.33	21.20	19.20	14.56	16.87
Methanolic	4.00	23.78	21.22	19.76	24.34	21.44	27.23	21.54
Pet. Ether	4.00	17.62	14.36	19.56	15.35	21.27	18.76	16.35
Chloroform	4.00	14.54	14.46	14.22	15.78	16.87	14.82	14.71
Water	4.00	14.64	15.23	19.43	15.66	18.25	14.32	15.66
<i>Artocarpus heterophyllus</i> (Kathal)								
Hexanoic	4.00	19.51	18.61	17.87	26.72	20.43	19.36	16.30
Methanolic	4.00	22.41	20.19	19.33	23.60	21.23	26.43	20.77
Pet. Ether	4.00	15.66	16.73	19.10	15.40	22.54	18.22	15.84
Chloroform	4.00	13.46	14.28	15.24	14.68	14.20	13.86	16.80
Water	4.00	14.22	21.24	13.82	14.24	15.62	16.22	14.74
Antibiotics								
Tetracycline	10.0	7.46	12.13	13.24	14.86	7.22	10.61	12.33
Ampicillin	10.0	8.77	9.23	10.35	14.21	9.20	13.44	12.36
Ciprofloxacin	10.0	8.16	12.82	13.82	13.21	7.24	12.23	11.17

*Control no antibiotic, *strength of activity is presented as resistant (0.7 mm), intermediate (>12 mm) and susceptible (>18 mm).

tex fractions were found susceptible to both gram-positive and gram-negative bacteria. Bacterial pathogens including Gram-positive bacteria such as *Lactobacillus acidophilus*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus* and Gram-negative bacteria *Klebsiella pneumoniae*, *Escherichia coli* have shown susceptibility both in presence of solvent and aqueous extracts. In various bioassays solvent extracts have shown very low MIC value than broad spectrum antibiotic drugs that indicates higher susceptibility in latex samples. In all the latex extracts an overall MIC value was obtained that ranges from 0.14 - 2.88 µg/ml. Among all the latex extracts methanolic extract has shown higher susceptibility to bacterial strains followed by hexanoic, petroleum ether and chloroform extract. Methanolic extracts from all different plant species have shown lowest MIC value that was in a range of 0.122 - 0.245 µg/ml (Table 1). It was also noted that solvent extracts have shown higher susceptibility in comparison to water extracts as the MIC and MBC values obtained in solvent extracts were found significantly lesser than the water extracts. *Diospyros melanoxylon* (Tendu), methanolic extract has shown higher MIC value 0.070 µg/mL against *K. pneumoniae*, while hexanoic extract *i.e.* 0.48 µg/mL against *S. pneumoniae*. Its water extract has shown MIC value 0.72 µg/mL against *Kleb-*

siella pneumoniae, and *Streptococcus pneumoniae* (Table 1). *Terminalia bellirica* (Wahera), methanolic extract has shown higher MIC value against *E. coli* and *M. luteus* 0.14, and 0.24 µg/mL while water extract has shown lowest MIC value 0.72 µg/mL against *E. coli* and *Lactobacillus acidophilus* (Table 1). *Ficus glomerata* (Gular) methanolic extract has shown higher MIC value in a range of 0.14 µg/mL - 0.24 µg/mL against *Lactobacillus acidophilus* (Table 1). *Phyllanthus emblica* (Awla) methanolic extract has shown higher MIC value against *Klebsiella pneumoniae* and *Streptococcus pneumoniae* 0.14 µg/mL (Table 1). Its water extract has shown lowest MIC value 0.36 - 0.14 µg/mL against *Staphylococcus aureus* and *Lactobacillus acidophilus*. Methanolic extract of *Thevetia neriiifolia* (Kaner), has shown lower MIC value 0.14 µg/mL and 0.24 µg/mL against *E. coli* and *S. aureus*. Its water extract was found active against three bacterial strains as the MIC values obtained are very low *E. coli*, *B. cereus* and *Lactobacillus acidophilus* 0.72 µg/mL. *Calotropis procera* has shown very high antimicrobial susceptibility as the MIC value obtained was least in all cases. It was ranging from 0.14 - 0.56 µg/mL against different bacterial strains. Its water extract has shown good antimicrobial activity as the MIC value was ranging from 0.36 - 1.44 µg/mL (Table 1). MIC values obtained in case of broad spectrum antibiotic drugs tetracycline, ampicillin and ciprofloxacin were higher than the latex samples. These were ranging from 0.8 - 6.40 µg/mL against different bacterial strains (Table 1). Presence of this antibacterial and antifungal activity against pathogenic bacteria and fungi in plant latexes may be due to presence of certain chemical components such as acetogenins, alkaloids, acids, terpenes, enzymes, protein, resins, tannins, sterols, sugars, oils, glycolipids and cysteine proteases. The leaves of *J. curcas* contain apigenin, vitexin and isovitexin, which along with other factors show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [10].

Furthermore, MBC (minimum bactericidal concentration) values of solvent and aqueous extracts were also determined. *Spondias dulcis* (Amra) methanolic extract has shown lowest MBC value 0.48 µg/mL against *S. pneumoniae*. It was quite higher in water extract as 2.88 µg/mL in each case. Similar MBC value was also obtained in *Diospyros melanoxylon* (Tendu), water extract. *Diospyros melanoxylon* methanolic extract has shown lower MBC value in *K. pneumoniae*, *E. coli*, *M. luteus* and *S. aureus* i.e. 0.56 µg/mL (Table 1). *Terminalia bellirica* (Wahera), methanolic extract has shown MBC value against *E. coli* and *M. luteus* 0.24 and 0.48 µg/mL while water extract has shown lowest MIC value 2.88 µg/mL against all bacterial strains (Table 1). *Ficus glomerata* (Gular) methanolic extract has shown lower MBC value 0.28 - 0.56 µg/mL against *Klebsiella pneumoniae*, *E. coli*, and *Lactobacillus acidophilus* (Table 1). Its water extract has shown MBC value 1.44 µg/mL against *K. pneumoniae*. *Phyllanthus emblica* (Awla) methanolic extract has shown MBC value 0.56 µg/mL against *Klebsiella pneumoniae*, *E. coli* and *Streptococcus pneumoniae* 0.14 µg/mL (Table 1). Its water extract has shown lowest MBS value against *Streptococcus pneumoniae*, *Staphylococcus aureus* and *L. acidophilus* 1.44 µg/mL. Methanolic extract of *Thevetia neriiifolia* (Kaner), *Calotropis procera* (Ak) and *Artocarpus heterophyllus* (katal) showed lower MBC values ranging from 0.56 - 2.44 µg/mL while water extract from these plants have shown MBS MBC values between 0.72 - 2.88 µg/mL (Table 1). Aqueous extracts in all the cases have shown lower MBC value ranging from 0.10 - 0.66 µg/mL. It has shown very high susceptibility to all the bacterial strains tested in the antimicrobial bioassays. *In vitro* data obtained reveals that latex samples from ten plant species were highly susceptible to both bacteria. Among eight plant species *Calotropis procera* (Ak), and *Carica papaya* (Papita), have shown highest susceptibility to different microbial strains as lowest MIC values were obtained in latex extracts. MIC values obtained in methanolic extracts were between 0.14 - 0.56 µg/mL. Similar MIC value was also obtained in *Ficus benghalensis* (Bargad) *in vitro* antimicrobial assays (Table 1).

Similar, antibacterial activity is reported in latexes of *A. ochroleuca* [2], *Hyaenanche globosa* (Euphorbiaceae) [28], *Hancornia speciosa* Gomes (Apocynaceae) against *Klebsiella*, *Pantoea*, *Enterobacter* and *Burkholderia* [7]. *Hevea brasiliensis* latex shows antifungal activity against *Trichosporum cutaneum* and *Cryptococcus neoformans* [5]. It also exhibits a good activity against multiple drug resistant *Staphylococcus aureus* (NCTC 11994), *Salmonella typhimurium* (ATCC 1255) and *Candida albicans* (ATCC 10231) [11]. When compared MIC values obtained in broad spectrum antibiotics against all seven bacterial strains were approximately 3.0 times higher than the latex extracts. It indicates that bacterial strains have developed resistance against these antibiotics. It was further confirmed by 2.5 times higher MBCS obtained in these antibiotics in *in vitro* bioassays.

The crude latex of *A. ochroleuca* exhibited a potent antibacterial effect against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Bacillus subtilis* [2]. Similarly, a South African plant; *Hyaenanche globosa* (Euphorbiaceae) showed poisonous activity due to presence of some toxic compounds [28]. Latex of *Aloe harlana* Reynolds shows antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrum* [26]. Methanolic extract of three *Salvia* species [29] have shown strong antimicrobial activity [30].

The ethanolic extract of the fruits of *H. globosa* (F.E) contain monophene which showed inhibitory and cytotoxic activity in HeLa cells' and other cancer cell lines [31]. Similarly, Tutin and hyenanchin isolated from *H. globosa* have shown significant ($P < 0.01$) inhibition on cell viability/proliferation [28]. Latex of *Jatropha curcas* is used externally to treat infection, piles and sores among the domestic livestock. The leaves of *J. curcas* contain apigenin, vitexin and isovitexin, which along with other factors show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [10]. Similarly, latex of *Argemone ochroleuca* Sweet has shown antibacterial activity against *Bacillus subtilis*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Escherichia coli*, and *Staphylococcus aureus* [2]. Latex from *Hancornia speciosa* Gomes (Apocynaceae) has shown activity against *Klebsiella*, *Pantoea*, *Enterobacter* and *Burkholderia* [7] while *Hevea brasiliensis* latex shows antifungal activity against *Trichosporum cutaneum* and *Cryptococcus neoformans* [5]. It also exhibits a good activity against multiple drug resistant *Staphylococcus aureus* (NCTC 11994) and *Salmonella typhimurium* (ATCC 1255) and *Candida albicans* (ATCC 10231) [11]. Similarly, antibacterial activity is reported in *C. procera* by Jain *et al.*, (1996) [8]. Hexane extract of Tunisian caprifig latex from the unripe fruit of *Ficus carica* showed strong anti microbial activity [32].

Besides determination of MIC and MBC values the effectiveness of all fruit latex extracts was confirmed by filter paper disc diffusion bioassay. After 24 hrs of in vitro Kirby Bauer test growth inhibition were measured in tests and controls. However, IZD obtained in presence of each latex extract was considered as final bactericidal marker. These are displayed in **Table 2**. On the basis of size of growth inhibition zone pathogens were divided in to three categories *i.e.* resistant (>7), intermediate (>12) and susceptible > 18 mm respectively. The zone of inhibition above 7 mm was considered as positive results. In present study broad-spectrum antibiotics have shown marginal activity or intermediate effect while latex samples have shown IZD more than 13 mm in each case. It shows that latexes contain pharmaceutically important active principles which are solvent extractable. Methanolic extract of *Spondias dulcis* (Amra) methanolic extract has shown highest inhibition zone diameter 28.13 mm against *K. pneumoniae* followed by *B cereus* 25.51 mm, *M luteus* 23.2 mm, *L acidophilus* 21.2 mm. In its hexane extract highest inhibition zone diameter 20.36 mm against *S. aureus*, Petroleum ether extract against *M luteus* 19.76 mm, chloroform against *B cereus* 16.10 mm. Water extract has shown inhibition zone diameter 15.32 mm against *E coli* (**Table 2**). Almost similar activity was reported in methanolic extracts from all ten different species. An overall activity susceptibility was displayed from MIC and MBC and IZD in a order of methanolic < hexanoic < petroleum ether < chloroform < water. Similarly methanolic extract of *D melanoxylon* (Tendu) has shown inhibition zone diameter between 19.65 - 25.48 mm against all different pathogens subsequently. It has shown highest inhibition zone diameter 25.48 mm against *L. acidophilus* (**Table 2**). Its water extract has shown inhibition zone diameter 16.26 mm against *E. coli*. In all tests inhibition zone diameter obtained in presence of solvent extracts were higher in comparison to water extracts from all plant species screened. Furthermore, these extracts have shown significantly higher inhibition zone diameter 15 - 31.63 mm than the broad spectrum antibiotics as these were noted 7.46 - 14.86 mm in. From the experiments methanolic extract were found to be highly susceptible against all the bacterial strains. As their growth inhibition zone diameter was obtained between 17.6 - 31.63 mm in size (**Table 2**). This inhibition was observed as at 4- μ g concentration of each extract at 24 h in agar disc diffusion bioassay. It was also observed that solvent extracts have shown higher inhibition zone diameter in comparison to water extracts. It may be due to higher diffusion of latex components coated on filter paper discs.

Further, in susceptibility bioassays growth inhibition was found to be dose dependent. Similarly, increase in radial growth was dose dependent. The results indicate that the highest radial growth inhibition was obtained in methanolic extract *Ficus glomerata* and *Calatropis procera* at 4 μ g/mL in comparison to broad spectrum antibiotics which have shown intermediate radial growth inhibition zone diameter less than 13 mm (**Table 2**). It indicates that these antibiotics are worth less as the inhibition zone diameter was obtained near 7 mm which indicates no susceptibility to microbes or marginal activity. From results it is clear that latex components are potential inhibitors of bacteria growth in suspension and solid agar culture. Similar antimicrobial activity was found in *Croton bonplandinum* [33] [34] [10], *Juniperous oxycedrum* [26], *Himatanthus articulatus* [34], *Salvia* species [29], *Argemone ochroleuca* [2], *Carica papaya* [5], *Calatropis procera* [30], *Hevea brasiliensis* [35] and *Ficus carriaca* latex against resistant pathogens [3].

This activity in plant latexes is due to presence of various active components such as Alpha-D-mannosidase and N-acetyl-beta-D-glucosaminidase [5], dieterpenes [4], certain proteases [36] and chitinases [37]. Similar activity against phyto-pathogens is reported in latexes of *Calatropis procera* (CpLP), *Plumeria rubra* (PrLP), *Ca-*

rica candamarcensis (PIG10) and *Euphorbia tirucalli* (EtLP) due to presence of antimicrobial proteins [6]. Crofelmer isolated from *Croton lecheri* [23] and Cotinifolia from *Euphorbia cotinifolia* latex have shown high antimicrobial activity [38]. *Hevea brasiliensis* latex shows antimicrobial effects against oral micro-organisms [39]. Latex from *Jatropha curcas* and *Artocarpus heterophyllus* were found active against all seven bacterial strains microbes due to presence active principles of Latex from *Himatanthus succubae* exhibited Leishmanicidal activity [21]. Fruit chitinases in banana latex have shown significantly higher antimicrobial susceptibility to the antibiotics [40]. Similar antimicrobial activity is reported in *Calotropis procera* latex due to presence of Osmotin [41]. Procerain, a stable cysteine protease isolated from the latex of *Calotropis procera* and anthrone and chromone shows strong antimicrobial activity [11] [42]. Latex proteins prevent septic shock due to lethal infection by *Salmonella enterica serovar Typhimurium* [43]. *Hevea brasiliensis* on oral microorganisms [39], Saponins-rich fraction of *Calotropis procera* leaves elicit no antitrypanosomal activity [44], while synergistic effect was shown in of *Carica papaya* latex sap and fluconazole on *Candida albicans* growth [5]. *Himatanthus succuba* latex was found active against *Leishmania amazonensis*. [21]. Latex from Apocynaceae species showed antiplasmodial activity [45] while Euphorbia species showed antileishmanial and antitrypanosomal [46] [47]. More often, plant latex contain many proteolytic enzymes and other proteins which lyse bacterial cell wall, therefore and latex act heavily upon human and veterinary pathogens.

It was cleared from bioassays that plant latex is more bactericidal to the gram-positive bacteria. This may be due to three important reasons *i.e.* first latex components are more diffusible and of low molecular weight and solvent and water soluble. Second absence of lipo-polysaccharide layer, that function as an effective barrier against any incoming bio-molecule [48] [49]. There might be another possibility that latex may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to ion leakage from the cell [50] [51]. Higher diffusion also occurs due to hydrophilic nature of bacterial cell wall [52] that results an increase in antimicrobial properties of active components [3]. No doubt latexes show multiple biological activities such as cytotoxic [53], antiproliferative and antiplasmodial [54], anti-inflammatory [55], Fibrinolytic [56], Anti-helminthic [57], anti-viral [58] antitumor activity [59] and strong antifungal activity against mycelial fungi [35] [41]. It is due to presence of proteases, chitinases and Osmotin, diterpenes and saponins which play important role in plant defense [41] [60]. These components displayed higher antimicrobial potential that can work as strong antimicrobial agents for of chemotherapy [25] for multidrug resistant human pathogens [61].

In the present study fruit latex fractions from ten latex secreting plant species registered significantly higher growth inhibition than that of broad spectrum antimicrobial drugs. The MIC and MBC values obtained in these fractions were significantly lower than that of antibiotics of broad spectrum activity. In addition, in presence of latex extracts IZD obtained were found more than 14 mm. Highest inhibition zone diameter against *M. luteus* (33.2 mm) followed by *K. pneumoniae* (29.26 mm) and *L. acidophilus* (28.43 mm) was obtained in presence of methanolic extract of *Thevetia nerifolia*. Though, no report is available on presence of volatile components in plant latexes but very high diffusion activity obtained in pure latex samples at room temperature, showed association of some volatile action that may be more active against bacteria in comparison to crude latex. Therefore, major anti-bacterial action in fungi seems to be diffusion action of plant latex or inhibition of metabolism of fungal strain. However, from previous studies no report is available on its volatile action *in vitro*. However, plant latex can be used as antimicrobial agent without any side effect [46]. So far studies have been done number of medicinal plants have been explored for their antimicrobial activity with fewer side effects and reduced toxicity. Therefore, it can be concluded that latex components if purified might prove potent therapeutic agents, which could become possible only after identification of major latex constituents. However, it could be concluded that latex induced inhibition and cidal activity against bacterial and fungal strains is due to presence of significant active principles. No doubt, if latex components are identified these could be used as therapeutic agents against various pathogenic bacterial and fungal strains.

5. Conclusion

Latex extracts prepared from ten laticiferous Indian plant species have shown strong antibacterial and antifungal activity. It is due to presence various latex components such as proteins, proteases, chitinases, osmotin, alkaloids, glycosides, diterpenes and saponins which can act as potent therapeutic agents. Latex samples have shown higher growth inhibition and cidal activity against bacterial and fungal strains than the broad spectrum antibiotic drugs due to presence of significant active principles. No doubt, if latex components are identified these could

be used as multiple therapeutic purposes and food preservation applications.

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