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A comparative study of *Kigelia pinnata* fruit extracts in terms of antimutagenic potential and antimicrobial efficacy against antibiotic-resistant microbial strains

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In recent years, antibiotic-resistant microbes have become a serious concern which needs proper attention either to solve the problem or to find out the solution to treat it. In this study, antibiotic resistant strains of *Pseudomonas*, *Enterococcus*, and *Escherichia coli* (*E. coli*) were used to assess the antimicrobial potential of *Kigelia pinnata* fruit extract. Further, antimutagenic potential of *Kigelia pinnata* fruit extract was also assessed by Ames assay using *Salmonella typhimurium* strain TA 98 and TA 100. In antimicrobial assay, only chloroform, ethanol and hexane extract was found to produce clear zone diameter between 1.08±0.1 to 2.1±0.2 mm. Results of minimum inhibitory concentration revealed the effectiveness of chloroform extract on *Pseudomonas*, *Enterococcus* and *E.coli* at 1.8 mg/mL concentration. However, better antimicrobial activity was found with ethanol extracts at $2.1x10^{-2}$ mg/mL concentration revealing the effectiveness of the low dose of ethanol in killing the antimicrobial resistant strains. In the time-kill test method, chloroform extract of *K. pinnata* was found to be most effective in reducing 98-99% test microbial population at both dilutions in 30 min. Antimutagenicity test showed the equal potential of chloroform and ethanol extracted *Kigelia* fruit sample in reducing the number of revertants. *Kigelia* fruit extract (1000 µL) dose can reduce the mutagens at 5 µg/plate level but not at 10 µg/plate dose level. Further research will open the new scope in the field of development of herbal antimicrobials and antimutagenic compound for treating antibiotic-resistant microbes and cancer.

Keywords: Ames test, antimicrobial activity, Balam kheera, Plant extract, *Salmonella* mutagenicity assay

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Antimicrobial drugs are used to kill or stop the growth of microbes and treat microbes based diseases. These drugs, generally, control the spread of microbial infection in the body. However, over-use, misuse or incomplete course of the antimicrobial agents leads to the development of resistance in microbes. Occasionally, cross-resistance to two or more drugs occurs which is also mediated by over-use, misuse or incomplete course of antibiotics. The treatment of resistant microbes is a complicated task due to the non-responsiveness of microbes[1] and situation is more complicated if these microbes spread in the environment. First- line or second- line antibiotic therapy does not provide complete treatment coverage to resistant microbes. There is a dire need for the development of new antimicrobial agents with improved activity against such resistant microbes. Furthermore, newly developed method or solutions must show positive influence without side effects on the patients, in order to get acceptance.

Another problem related to human health is their exposure to mutagenic compounds which occur through environmental and occupational sources, food and water, as well as through cellular reactions causing damage to DNA due to nascent oxygen-derived free radicals. The mutations in the non-functional area of DNA do not show any effect in the cell and hence, called silent mutation. However, the mutations in the coding region show profound effect on gene expression, protein expression and its function which may cause death of the patient under chronic exposure[2].

Accidental and occupational exposure to these mutagens can also induce genetic inheritable diseases and cancer[3,4]. There is a need for developing solution with antimutagenic effect to nullify the action of mutagens. To combat carcinogenesis and controlling deleterious effects of mutagens, different plants are

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suggested as an effective intervention[5-6]. Medicinal plants, having antimicrobial properties[7-9], have been used to treat a variety of diseases since ancient times. These are the part of the traditional medicinal system worldwide. Plant extracts of many medicinal plants have been reported to possess antimutagenic phytochemicals that can be used to nullify mutagenic effect and cancer[4].

Keeping the above points in mind, the present study was conducted to find out the antimicrobial efficacy of *Kigelia pinnata* extract on antibiotic-resistant microbes along with its antimutagenic potential. *Kigelia pinnata*, also known as *Kigelia africana*, is a tropical tree which is an introduced plant species in India. The fruits, roots, wood, the bark of the stem and the leaves are used for medicinal purposes. Fruits cannot be used for edible purposes as it causes blisters in the mouth and on the skin. However, it is of medicinal importance in African folk medicine due to its use against dysentery, venereal diseases. It is also used for topical application on wounds and abscesses[10]. The bark of *Kigelia* is used as analgesic[11] along with other applications in rheumatic arthritis, dysentery, venereal diseases, malaria, pneumonia, diabetes[12] and toothache. It is also used to treat the diseases caused by worms like ringworm, tapeworms[13].

The antibacterial activity of aqueous, methanol and chloroform extracts of *Kigelia africana* bark has been reported. Methanolic extract of the bark of *Kigelia* was reported to be effective on *Salmonella typhi* and *Proteus vulgaris*[13]. The efficacy of *Kigelia* plant extract against bacterial and fungal pathogens has also been reported in the literature[13,14-16]. However, the effect of *Kigelia pinnata* has not been checked on antibiotic-resistant microbes. Henceforth, in the present work, effectiveness of *K. pinnata* extracts against antibiotic resistant bacteria has been checked along with its antimutagenic potential using *S. typhimurium* as experimental system.

Material and Methods

Collection and processing of plant material

Kigelia pinnata was identified on the basis of its morphological characteristics. The height of tree was approx., 20 m with woody stem. The wood was pale brown or yellowish, undifferentiated and not prone to cracking. The leaves were 30-50 cm long found in whorls of three. The flowers hang down from the branches on 2 to 6 m long pedicel. The bell-shaped, large flowers are reddish or purplish which are produced in panicles. The fruit is 30 to100 cm long with light brown peel and woody walls. The seeds are ovoid and embedded in the centre along with fibrous pulp. Plant samples were deposited in the Department of Botany, University of Rajasthan, Jaipur with accession number RUBL212707. Figures of *Kigelia pinnata* fuits and leaves shown as Supplementary Figures 1-3.

The fruit samples were collected from the tree in July 2018 and cut in small pieces. The samples were dried in hot air oven at 45°C for 48 h. After drying, samples were ground in grinder to obtain them in powdered form. The powder was filtered using sieve 0.5 mm to maintain uniform particle size. This powder was sealed in polythene bags and kept at room temperature and further used for the preparation of extract.

Selection of microbial strains

Clinical microbial isolates were provided by Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur. The culture was revived using nutrient agar and antimicrobial resistance test was performed using antibiotics like penicillin, ampicillin, erythromycin, streptomycin and tetracyclin. Antibiotic-resistant strains of *Pseudomonas, E.coli* and *Enterococcus* were selected for the assessing the antimicrobial efficacy of *Kigelia Pinnata* extracts in the present study.

Preparation of fruit extract in different solvents by soxhlet method

The dried fruit powder 10 g was used to prepare extract in 300 mL of 100% i.e., pure chloroform, ethanol, petroleum ether, water and hexane by Soxhlet method. All samples were heated not above the boiling point of solvent. The soxhlet was run till the fruit powder was completely decolorized. The extract was concentrated by drying process at 40 to 50 °C in rotary vacuum shaker evaporator. The concentrated extracts were taken out and transferred to microfuge tubes which were kept at dry bath for complete drying. The extracted and dried material was re-dissolved in known amount of solvent. Again temperature was not allowed to exceed the boiling point. This step was introduced to maintain the uniform concentration.

Antimicrobial assays

The antimicrobial assays were performed by disc diffusion method. Minimum inhibitory (MIC)

concentration of *Kigelia pinnata* extracts was also calculated to find out the effective dose. Time-kill test method was performed by the direct exposure of *Kigelia pinnata* extract with the microbes. This will give us an appropriate idea of antimicrobial activity.

Disc diffusion method

In this method, discs made up of whatman filter paper were impregnated in pure ethanol, chloroform, petroleum ether, hexane and aqueous derived whole fruit extracts of *Kigelia pinnata*. This was followed by air drying of disc at room temperature and then plating on nutrient agar containing plates followed by incubation at 37°C for 24-48 h. The zone of inhibition of microbes was measured.

Time-Kill test method

This is the most appropriate method for determining the bactericidal effect of herbal extract[17,18]. This method reveals a time-dependent or a concentration-dependent antimicrobial effect. The method mentioned by Coudron and Stratton[16] for screening the antimicrobial activity was followed in the study. In this method, 10 mL of Muller Hinton broth was poured in sterile tubes and inoculated with a colony of *Pseudomonas, Enterococcus* and *E. coli* and incubated overnight (18 h) at 37°C. *Pseudomonas, Enterococcus* and *E. coli* culture was found to have $2.3x10^6$ cfu/ml, $1.5x10^6$ cfu/mL and $1.1x10^6$ cfu/mL, respectively. In other sterile eppendorf tubes, 10-fold dilution of the ethanol, chloroform derived *Kigelia pinnata* fruit extract was prepared in their respective solvents. The selection of dilution of *Kigelia* fruit extract was guided by the results of minimum inhibitory concentration[19]. About 1 ml pure extract, 10^{-1} and 10^{-2} dilution of *Kigelia pinnata* fruit extract were mixed with 2 µL of the culture of *Pseudomonas, Enterococcus,* and *E. coli*. In growth control, the culture was added to normal saline. To calculate the survival rate, the samples were kept for incubation at 37°C and withdrawn after 30 min and 20 µL was plated on Mueller Hinton agar (MHA) plates. Then, the percentage of reduction of microbial count was calculated by counting the colonies appeared on MHA plates. The efficacy of *Kigelia* fruit extract was calculated by determining the percent decrease in the number of colony forming units appeared on the plate after 30 min with respect to their initial count.

Antimutagenic assay

To detect the presence of mutagenic compounds in the sample, *Salmonella*/microsome assay (Ames test) was developed by Maron and Ames[20]. The same test was used with some modifications[21] to assess the antimutagenic potential of *Kigelia* fruit extract[22]. The antimutagenic assay was performed using the preincubation procedure. The strains of *Salmonella typhimurium* TA 98 and TA 100 were purchased from Microbial Type Collection Center (MTCC), Chandigarh. These cultures were transferred separately in 10 mL broth and incubated for 18-20 h to get 2×10^9 cells/mL concentration. 250 µL of each culture was added to the mixture of 250 µL mutagen (sodium azide: 5 and 10 µg/plate; 2-nitrofluorene: 5 and 10 µg/plate) and 500 µL of histidine/biotin solution, 500 µL phosphate buffer and 1000 µL of different dilutions of plant extract i.e., 100 µL, 200 µL, 500 µL and 1000 µL (undiluted plant extract). The experiment was also conducted by adding S9 mix i.e., having hepatic fraction of mouse which gives an idea about *in vitro* effect of mouse liver enzymes on the strength of mutagens. The liver enzymes may increase or decrease the mutagenicity. As *Salmonella* strains reveal the level of mutagenicity in prokaryotic system, incorporation of S9 provides the idea of mutagenicity in eukaryotic system. The mixture of culture, mutagens and fruit extract is plated on the minimal media and incubated at 37°C for 48 h. Further, the number of revertants appeared on the plate were counted. In this assay, sodium azide and 2-nitrofluorene were used as positive control for *S. typhimurium* TA 100 and TA 98 respectively[22].

Statistical analysis

The experiment of antimicrobial activity and minimum inhibitory concentration were repeated thrice. Mean and standard deviation was calculated for clear zone diameter of antimicrobial assay. Statistical analysis was carried out by one way ANOVA using origin 50 software. The significant difference in the antimicrobial activity was determined at $p<0.05$ level of significance.

Data analysis of antimutagenic potential

Antimutagenic potential of *Kigelia pinnata* fruit extract was analyzed by two methods: statistical and non-statistical methods. In the statistical method, means of the number of revertants appeared on three plates were calculated. The mean number of revertants was used to calculate the percent reduction of number of revertants using the following formula:

Percent reduction in the number of revertants =

(NRPC-NRPP)/NRPC x 100

In the formula, NRPC is number of revertants appeared on the positive control plate (mutagen is added without adding *Kigelia* fruit extract) and NRPP number of revertants appeared on the plate having *Kigelia* fruit extract.

In the present study, non-statistical analysis of mutagenicity has also been done by calculating the mutagenicity ratio according to two-fold rule. The rule specifies that doubling of spontaneous reversion rate at one or two mutagens concentration considered a positive response[23]. Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity which is shown by "+" sign otherwise "-" sign.

Results and Discussion

In this study, antimicrobial activity of ethanolic, aqueous, chloroform, hexane and petroleum ether extract of *Kigelia* fruit on the antibiotic-resistant strain of *E. coli*, *Enterococcus,* and *Pseudomonas* sp. was observed. In the initial test, antibiotic resistance was observed, and resistant strains were selected for assessing the efficacy of *Kigelia* extract. *Pseudomonas* sp. was found to be resistant for erythromycin, tetracyclin, ampicillin, and penicillin (Fig. 1a). Similarly, *E.coli* was found to be resistant for penicillin, tetracyclin, and ampicillin (Fig. 1b) while *Enterococcus* sp. was found to be resistant only for penicillin antibiotic (Fig. 1c).

Antimicrobial efficiency

Antimicrobial efficacy of different extract of *Kigelia pinnata* is shown in Fig. 2. The clear zone in the plate surrounding the extract laden disc revealed the efficacy of whole fruit extracts of *Kigelia pinnata*. The clear zone diameter for all the extracts was found to be in the range of 1.08±0.1 to 2.1±0.2 mm ($p>0.05$). Chloroform extract of *Kigelia pinnata* fruit was found to be effective with antibiotic-resistant strain of *Pseudomonas* (Fig. 2a), *Enterococcus* (Fig. 2b) and *E. coli* (Fig. 2c). However, ethanol and hexane extracts were found to be effective with *Enterococcus* and *E. coli*. Petroleum ether extract was found to be effective only on *Enterococcus* sp. while aqueous was not found to be effective on any strain. The highest activity i.e., clear zone diameter (2.1 mm) was found to be with ethanol and n-hexane extract ($p>0.05$) (Fig. 2d). The clear zone diameter did not vary significantly for all the strains and extract of *Kigelia* fruit. The efficacy of chloroform extract was non-significantly lower than the ethanol and hexane extract however, it was found to be effective with all the microbial strains. In this study, aqueous extract was not found to be effective on antibiotic-resistant test strains. This finding was in contrast with the earlier reports in which aqueous extract was found to be effective on *Bacillus subtilis. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans*[24] revealed the ineffectiveness of the aqueous extracts on the antibiotic resistant strains.

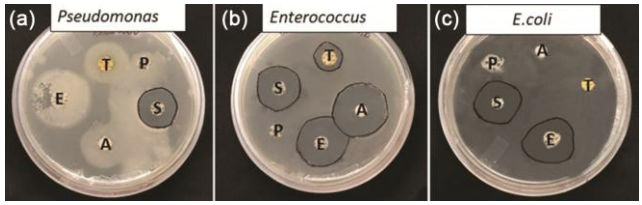

Fig. 1 — Antibiotic resistant strains of *Pseudomonas, Enterococcus,* and *E. coli.* (a): Erythromycin (E), tetracyclin (T), ampicillin (A) and penicillin (P) resistant *Pseudomonas.* (b): Penicillin (P) resistant *Enterococcus*. (c): Penicillin (P), tetracyclin (T), and ampicillin (A) resistant *E. coli.*

Minimum inhibitory concentration (MIC) of *Kigelia* fruit extract

Results of MIC are shown in Fig. 3. This test was not performed with aqueous extract of *Kigelia* as it was found to be ineffective in the antimicrobial test against *Pseudomonas, E. coli* and *Enterococcus* sp. Similar to the antimicrobial efficacy results, petroleum ether was found to be effective only against

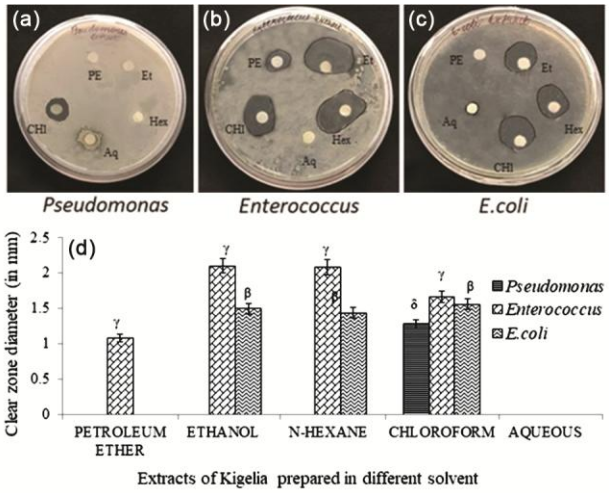

Fig. 2 — Antimicrobial activity of *Kigelia* extract on *Pseudomonas* (2a), *Enterococcus* (2b), *E.coli* (2c). Antimicrobial activity of *Kigelia* extract prepared in petroleum ether (PE), ethanol (Et), hexane (Hex), chloroform (CHl), Aqueous (Aq) solvent as measured by clear zone diameter (2d).
$^{\gamma}$: $p>0.05$ Other solvents vs Aqueous extract for *Enterococcus*
$^{\beta}$: $p>0.05$ Other solvents vs Aqueous extract for *E. coli*
$^{\delta}$: $p>0.05$ Other solvents vs Aqueous extract for *Pseudomonas*

Enterococcus sp. (Fig. 3d). 100% chloroform extracted *Kigelia* fruit extract was found to be effective against *Pseudomonas* sp., *Enterococcus* sp. and *E. coli* (Fig. 3b). Nevertheless, it is lesser effective than ethanol (Fig. 3a) and hexane extract (Fig. 3c) as revealed by clear zone diameter (Fig. 3e). Clear zone diameter of neat chloroform extracts (100%) (control) with *E. coli* and *Enterococcus* sp. was 0.8 mm and 0.6 mm, respectively while 100% ethanol and hexane extract (control) showed 1.7 to 2.1 mm with *Enterococcus sp. and E. coli* ($p<0.05$, $p<0.01$). In the case of pure ethanolic extract, minimum inhibitory concentration was $2.1x10^{-2}$ mg/ml at 10^{-4} with *Enterococcus* (1.06 mm clear zone diameter) and *E. coli* (0.86 mm clear zone diameter) (Fig. 3e). Similarly, the minimum inhibitory concentration of hexane extract of *Kigelia pinnata* was $1.9x10^{-2}$ mg/mL at 10^{-4} dilution with *Enterococcus and E. coli* as revealed by clear zone diameter i.e., 0.3 and 0.4 mm, respectively. In the case of chloroform extract of *Kigelia pinnata*, minimum inhibitory concentration was 1.8 mg/mL at 10^{-2} dilution with *Enterococcus* sp. and 10^{-3} with *E. coli*.

Results of this study revealed that chloroform extracted *Kigelia* fruit extract (180 mg/mL) was effective on all antibiotic- resistant microbes i.e., *E. coli, Enterococcus* sp. and *Pseudomonas* sp. However, ethanolic extract of fruit of *Kigelia pinnata* was found to be effective at minimum inhibitory concentration $2.1x10^{-2}$ mg/mL (10^{-4} dilution) on antibiotic resistant *E. coli* and *Enterococcus* sp. The

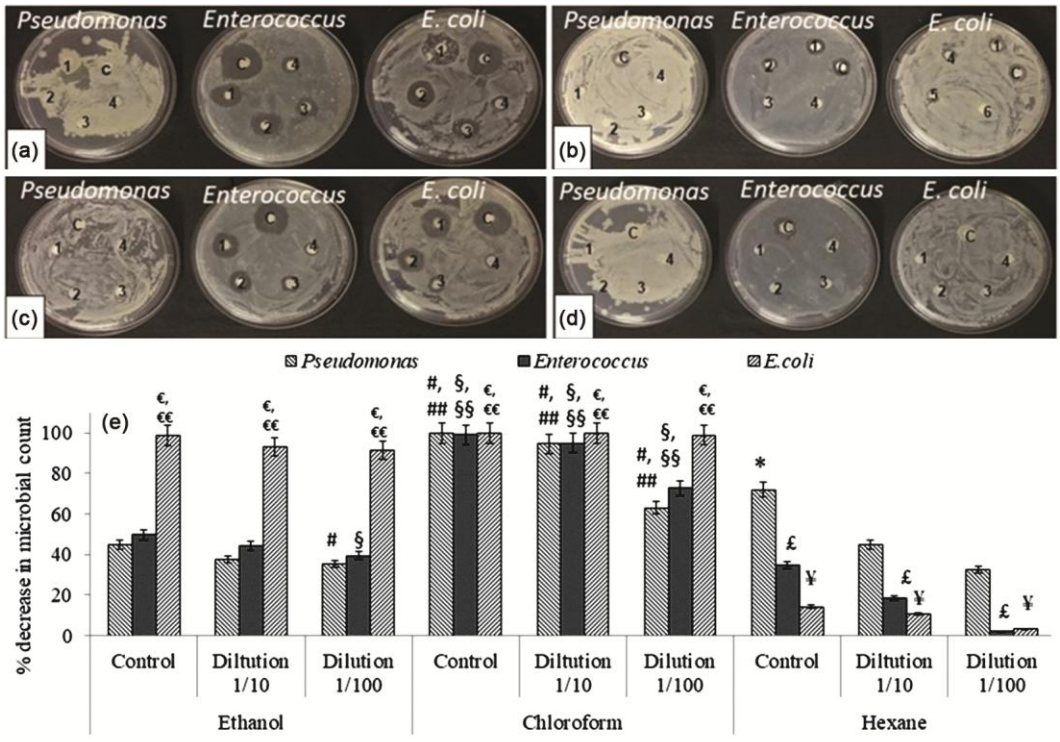

Fig. 3 — Minimum inhibitory concentration (MIC) of *Kigelia pinnata* fruit extract prepared in ethanol, chloroform, hexane, petroleum ether (PE) at dilution 1/10 (10^{-1}), 1/100 (10^{-2}), 1/1000 (10^{-3}) and 1/10000 (10^{-4}). Plate (A): MIC with ethanol extract; Plate (B): MIC with chloroform extract; Plate (C): MIC with hexane extract; (D): MIC with petroleum ether. In all plates, C: Control (pure extract); 1: 10^{-1} dilution; 2: 10^{-2} dilution, 3: 10^{-3} dilution, 4: 10^{-4} dilution. Plate E: Percent reduction in microbial count by ethanolic, choloroform, hexane extract at dilutions 1/10 (10^{-1}), 1/100 (10^{-2}).

§: $p<0.05$ Other extract vs Hexane extract for *Enterococcus*
€: $p<0.05$ Other extract vs Hexane extract for *E. coli*
#: $p<0.05$ Other extract vs Hexane extract for *Pseudomonas*
§§: $p<0.01$ Other extract vs Hexane extract for *Enterococcus*
€€: $p<0.01$ Other extract vs Hexane extract for *E. coli*
##: $p<0.01$ Other extract vs Hexane extract for *Pseudomonas*
*: $p<0.05$ Other extract vs ethanol extract for *Pseudomonas*
£: $p<0.05$ Other extract vs ethanol extract for *Enterococcus*
¥: $p<0.05$ Other extract vs ethanol extract for *E. coli*

previous studies conducted with *Kigelia pinnata* reported that the organic extract possess greater antimicrobial activity than aqueous extract due to the extraction of antibacterial substances in polar or non-polar organic solvent[22]. Jeyachandran and Mahesh[10] reported the effectiveness of methanolic extract of bark of *Kigelia africana* (Lam) on a variety of Gram-positive and Gram-negative microbes. Grace *et al.*[13] reported the presence of palmitic acid in the fruits of *Kigelia africana* which causes inhibitory effect on many Gram-positive and Gram-negative bacteria such as non-resistant *B. subtilis*, *E. coli*, *K. pneumoniae* and *S. aureus.* In this study, ethanolic extract of *Kigelia pinnata* was found to be effective with antibiotic-resistant *Enterococcus* sp. and *E. coli* at $2.1x10^{-2}$ mg/mL which is comparable with the results obtained by Grace *et al.*[13] on non-resistant microbes.

Time-kill test method

Time-kill test method was performed for control (pure extract) and 10^{-1} and 10^{-2} dilution of ethanol, chloroform, and hexane extract of *Kigelia pinnata*. Petroleum ether and aqueous extract were not used for time-based killing assay as these were ineffective as revealed by the results of minimum inhibitory concentration and antimicrobial assay. In the control plate, initial count of *Pseudomonas*, *Enterococcus and E. coli* was 2262, 2150 and 1332 cfu/mL, respectively.

In the case of ethanol extract, 10^{-2} dilution (concentration 2.1 mg/mL) was found to be effective in reducing 95-99% population of *E. coli* in 30 min. 35-50% (Fig. 4) reduction in the population of *Enterococcus* was observed at 10^{-1} (concentration 21 mg/mL) and 10^{-2} dilution (concentration 2.1 mg/mL) of ethanolic extract. Similar reduction was observed in the microbial count of *Pseudomonas* sp.

Pure chloroform extract (control, concentration 180 mg/mL) reduced 99% population of *Pseudomonas*, *Enterococcus* and *E. coli* and 10^{-1} dilution (concentration 18 mg/mL) was found to reduce 95-99% population of *Enterococcus*, *Pseudomonas* and *E. coli* in 30 min (Fig. 4). However, 10^{-2} dilution of chloroform extract (concentration 1.8 mg/mL) reduced 62, 72 and 98% microbial population of *Pseudomonas, Enterococcus* and *E. coli* in 30 min, respectively. Pure hexane extract of *Kigelia pinnata* (concentration 190 mg/mL) reduced 75% population of *E. coli*, 14% population of *Pseudomonas* and 35% of *Enterococcus* in 30 min. 10^{-1} dilution of hexane extract (concentration 19 mg/mL) was found to reduce only 44% population of *E. coli* and 10-18% reduction of *Pseudomonas* and

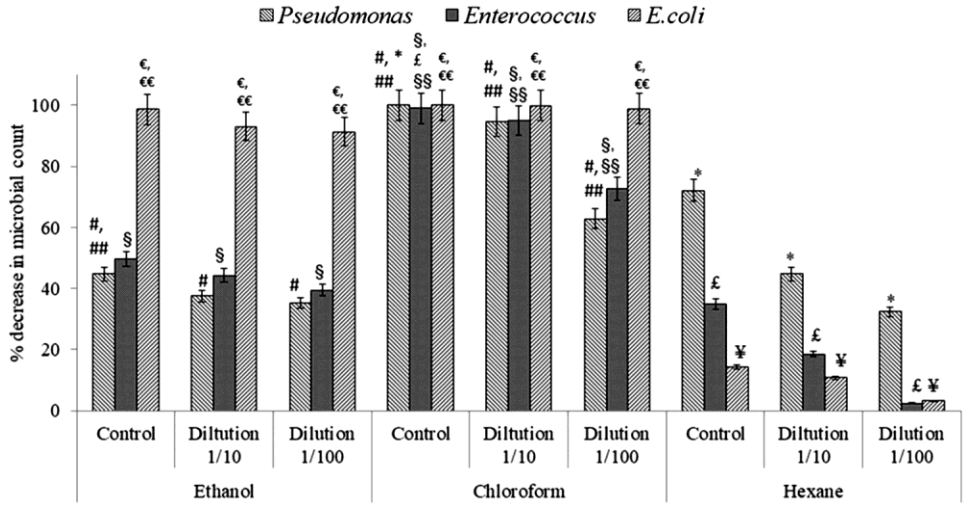

Fig. 4 — Time-kill test method shows percent decrease in the colony forming units with ethanol, chloroform, and hexane extracts.
§: p<0.05 Other extract vs Hexane extract for *Enterococcus*
€: p<0.05 Other extract vs Hexane extract for *E. coli*
#: p<0.05 Other extract vs Hexane extract for *Pseudomonas*
§§: p<0.01 Other extract vs Hexane extract for *Enterococcus*
€€: p<0.01 Other extract vs Hexane extract for *E. coli*
##: p<0.01 Other extract vs Hexane extract for *Pseudomonas*
*: p<0.05 Other extract vs ethanol extract for *Pseudomonas*
£: p<0.05 Other extract vs ethanol extract for *Enterococcus*
¥: p<0.05 Other extract vs ethanol extract for *E. coli*

Enterococcus. Dilution 10^{-2} of hexane (concentration 1.9 mg/mL) reduced the microbial population, however, was not very effective. Conclusion drawn out from the time-kill test method was that the effectiveness of pure chloroform extracts of *Kigelia* in reducing the microbial count to 99% in 30 min only. 10^{-1} dilution chloroform extract with concentration 18 mg/mL was also found to be effective in reducing microbial population of *Pseudomonas, E. coli and Enterococcus* (Fig. 4).

In time-kill test method, *E. coli* was found to be equally affected by pure ethanol and chloroform extracted samples. 10^{-2} dilution of chloroform extract (1.8 mg/mL) was also found to be effective in reducing the microbial count of *Pseudomonas, Enterococcus* and *E. coli*. MIC revealed the effectiveness of chloroform and ethanol extracts on *Enterococcus and E. coli* but not on *Pseudomonas*. In contrast to this, in the time-kill test method, direct exposure of microbial culture to the plant extract revealed the effectiveness of ethanolic extract with respect to control, in percent decrease in *Pseudomonas* count to 10^{-2} dilution (2.1 mg/mL concentration).

Time-kill test method is reported as the most effective method to evaluate anti-microbial efficacy of herbal formulations[26,27]. In contrast to the MIC results, chloroform extract was found to be more effective than ethanolic extract of fruits of *Kigelia pinnata* in reducing the microbial count of antimicrobial resistant strains i.e., *Pseudomonas, Enterococcus* and *E. coli*. The possible reason of this is the diffusibility of herbal extract through agar containing medium in the MIC test. However, the direct exposure of the extract to the microbial culture in time-kill test method showed promising results as compared to MIC. In the earlier reports, time-kill test method was reported to be an important approach to the primary selection of antimicrobial agent[19]. According to the time-kill test method, chloroform extract of *Kigelia pinnata* fruit was found to be most effective in the percent decrease in the microbial count at both dilutions in 30 min. There was no significant difference in the reducing the microbial population of antibiotic-resistant *Pseudomonas, Enterococcus* and *E. coli* showing the effectiveness of the chloroform extract in reducing the population of antibiotic resistant microbes.

Antimutagenicity assay

Time-kill test method showed the suitability of chloroform extract *Kigelia pinnata* fruit sample in reducing the microbial count of antibiotic-resistant strains of *E. coli, Pseudomonas* and *Enterococcus.* However, greater activity was reported for ethanolic extract with *E. coli* and *Enterococcus* sp. Therefore, antimutagenic potential of these two extracts i.e., ethanolic and chloroform extract was assessed and depicted in Fig. 5.

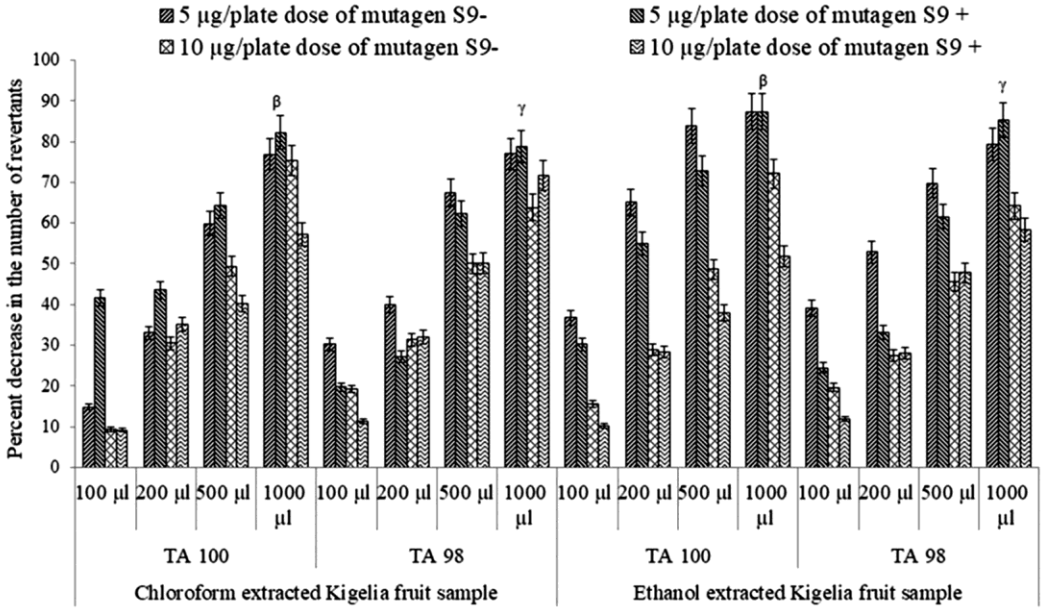

Fig. 5 — Antimutagenic effect of *Kigelia* fruit extract is shown in terms of percent decrease in number of revertants ($p>0.05$).
$^{\beta}$: ($p>0.05$) Chloroform extract vs. ethanolic extract for *S.typhimurium* TA 100 strain
$^{\gamma}$: ($p>0.05$) Chloroform extract vs. ethanolic extract for *S.typhimurium* TA 98 strain

In this study, *Salmonella typhimurium* strain TA 98 and TA100 were used to detect the frame shift and base pair mutagens, respectively in the sample. Figure 5 shows the percent reduction in the number of mutagens at 5 µg/plate and 10 µg/plate (dose level) with *Salmonella typhimurium* strain TA 98 and TA 100. The number of revertants appeared on the plate having at 10 µg/plate mutagen were higher as compared to 5 µg/plate. This shows that the number of revertants increased with increasing the dose level of mutagen.

At 5 µg/plate dose level of sodium azide, 100 µL chloroform extracted *Kigelia* fruit sample with 180 mg/mL concentration showed 14.7% reduction in the absence of S9 mix which increased to 41.6% reduction in the presence of S9 mix with *S. typhimurium* strain TA 100. As the dose of chloroform extracted fruit sample increased to 1000 µL, percent reduction in the number of revertants was 76.8% and 82.2% in the absence and presence of S9 mix, respectively. However, on incorporating 10 µg/plate of sodium azide, maximum reduction was 75.3% and 57% at 1000 µL dose of *Kigelia* fruit extract in the absence and presence of S9 mix, respectively (Fig. 5). Similarly, at 5 µg/plate dose level of 2-nitrofluorene, maximum percent reduction in revertants was 77% in the absence of S9 mix and 78.7% in the presence of S9 mix with *S. typhimurium* strain TA 98 at 5 µg/ plate dose of 2-nitrofluorene. On increasing the dose of 2-nitrofluorene (10 µg/plate), 63 and 71% reduction in the revertants was observed in the absence and presence of S9 mix, respectively. This showed that *Kigelia* extract becomes less effective if dose of mutagen is high.

1000 µL dose of ethanolic extract of *Kigelia,* at 5 µg/plate dose level of sodium azide reduced 87% revertants was observed in the presence and absence of S9 mix with *S. typhimurium* strain TA 100 which was not significantly higher ($p>0.05$) than the chloroform extract. Further, increase in the dose of sodium azide (10 µg/plate), reduction in the revertants was 72.1 to 51.8% in the absence and presence of S9 mix, respectively with *S. typhimurium* strain TA 100. Similarly, ethanol extract of *Kigelia* fruit sample (210 mg/mL) reduced the revertants up to 79.2% and 85.3% in the absence and presence of S9 mix, respectively with strain TA 98 at 5 µg/plate dose level of 2-nitrofluorene. In contrast to this, at 1000 µL dose of *Kigelia* fruit extract only 64 and 58% reduction was observed in the absence and presence of S9 mix, respectively at 10 µg/plate dose level of 2-nitrofluorene.

The results of this mutagenicity assay revealed that mutagenicity reduced maximum at 1000 µL dose of *Kigelia* plant extract with both the strains at 5 µg/plate and 10 µg/plate dose of mutagen in the presence and absence of S9 mix. This shows the efficiency of S9 mix (liver enzymes) in reducing the mutagenicity of mutagens with TA 98 and TA 100 strain ($p>0.05$). This anti-mutagenic effect of chloroform and ethanolic extract of *Kigelia* fruit was found lesser at 10 µL dose of mutagen because there is no significant difference in the number of revertants appeared on the plate. This shows that both ethanolic and chloroform containing extract are equally effective in reducing the mutagenicity of the mutagens i.e., sodium azide with *S.typhimurium* TA 100 and 2-nitrofluorene with *S.typhimurium* TA 98 ($p>0.05$).

Mutagenicity ratio, if more than 2 indicates the presence of mutagenicity and denoted by "+" signs and if below 2 indicates non-mutagenicity in the sample which is denoted by "-" sign (Table 1). At 5 µg dose level, 500 and 1000 µL of *Kigelia* fruit extract reduced the mutagenicity which is shown by lower mutagenicity ratio (below 2). Both base pair and frameshift mutagens were found to reduce and did not increase further on adding S9 mix indicating the role of liver enzymes in neutralizing the effect of mutagen. The highest reduction was observed at 1000 µL dose of *Kigelia* fruit extract. On increasing the dose of mutagen i.e., 10 µg/plate, mutagenicity did not reduce by either *Salmonella typhimurium* TA 98 or TA 100 strain by adding *Kigelia* fruit extract. No reduction in base pair and frameshift mutagens were observed further, S9 mix addition did not decrease it (Table 1).

The results of antimutagenicity test revealed the effective decrease of mutagenicity at 1000 µL dose of *Kigelia* fruit extract only at 5 µg/plate dose level of mutagen. In the previous reports, extracts of *Kigelia pinnata* was reported to be effective against melanoma and renal carcinoma cell lines[28]. According to Azuine *et al.*[29], *Kigelia* fruit extract has inhibitory effects on induced tumors. However, the cause of tumor inhibition is not reported. The results of this study showed antimutagenic effect of *Kigelia* fruit extract may neutralize the effect of mutagens and inhibit cancer growth.

Table 1 — Non-statistical analysis of anti-mutagenicity effect of *Kigelia pinnata* fruit extract by calculating mutagenicity ratio

	Strains of *Salmonella typhimurium*	Doses of Kigelia Fruit Extract	Doses of mutagen			
			5 µg/plate dose of mutagen		10 µg/plate dose of mutagen	
Chloroform extracted *Kigelia* fruit sample	TA 100	100 µL	S9- 3.97 (+)	S9 + 1.00 (-)	S9- 26.00 (+)	S9 + 14.27(+)
		200 µL	2.91(+)	0.94 (-)	19.69 (+)	9.89 (+)
		500 µL	1.34 (-)	0.23 (-)	14.07 (+)	9.04 (+)
		1000 µL	0.35 (-)	0.39 (-)	6.33 (+)	6.17 (+)
	TA 98	100 µL	5.81(+)	3.40 (+)	14.75 (+)	10.22 (+)
		200 µL	4.88 (+)	2.99 (+)	12.38 (+)	7.59 (+)
		500 µL	2.19 (+)	1.06 (-)	8.75 (+)	5.29 (+)
		1000 µL	1.25 (-)	0.16 (-)	6.03 (+)	2.58 (+)
Ethanol extracted *Kigelia* fruit sample	TA 100	100 µL	7.69 (+)	2.08 (+)	27.08 (+)	14.62 (+)
		200 µL	3.80 (+)	0.99	22.64 (+)	11.44 (+)
		500 µL	1.20 (+)	0.20 (-)	16.08 (+)	9.79 (+)
		1000 µL	0.73 (+)	0.44 (-)	8.28 (+)	7.38 (+)
	TA 98	100 µL	6.16 (+)	4.85 (+)	18.14 (+)	11.43 (+)
		200 µL	4.53 (+)	4.17 (+)	16.27 (+)	9.16 (+)
		500 µL	2.55 (+)	1.97 (-)	11.93 (+)	6.36 (+)
		1000 µL	1.44 (+)	0.13 (-)	7.52 (+)	4.87 (+)

(+) sign: Mutagenicity ratio > 2 which is the indication of mutagenicity
(-) sign: Mutagenicity ratio < 2 which indicates no mutagenicity

Conclusion

Antibiotic resistance among the microbial community is a major issue which needs to be properly addressed. Second is related to antimutagenic potential of plant extract. In the present study, antimicrobial effect and antimutagenicity effect of *Kigelia pinnata* fruit extract was studied. *Kigelia pinnata* fruit extract was prepared by using ethanol, aqueous, chloroform, petroleum ether and hexane. The antimicrobial assay against the antibiotic-resistant strains revealed the suitability of chloroform extracted *Kigelia* fruit sample in reducing the population of antibiotic-resistant strains of *E. coli*, *Pseudomonas* and *Enterococcus*. This was followed by ethanolic extract which showed highest activity against *Enterococcus* sp. and *E. coli*. Both these extracts were further used in assessing the antimutagenic potential using *S. typhimurium* strain TA 98 and TA 100. 1000 µl of both extracts were found to be equally effective in reducing the mutagenic effect of mutagens only at 5 µg/plate level. In the future, antibiotic-resistant microbes can be treated by using ethanolic or chloroform extracted *Kigelia pinnata* fruit extract. Then, effect of mutagenicity can be reduced by using the same extract of *Kigelia pinnata* fruit extract. Further research in this direction helps us in exploring the antimutagenic effect of *Kigelia pinnata* fruit extract. In the near-term, antimutagenic compounds can be isolated from the crude extract of *Kigelia pinnata* fruit.

Supplementry Data

Supplementry data associated with this article is available in the electronic form at http://nopr.nscair.res.in/jinfo/ijtk/IJTK_21(01)(2022)224-233_SupplData.pdf

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Conflict of Interest

There is no conflict of interest.

Authors' Contributions

Aayushi Jain- Conduction of experiment in laboratory and Data compilation; Harish Kumar- Guidance and revision of manuscript; Chintan Tripathi- Data compilation and graphs preparation; & Shweta Kulshreshtha- Research guidance, data compilation, Manuscript writing and revision.

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