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Evaluation of Antioxidant, Antibacterial and Phytochemical Properties of *Kalanchoe Pinnata, Kalanchoe Robusta* and *Kalanchoe Blossfediana*

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ABSTRACT

Extracts of *Kalanchoe pinnata* (KP), *Kalanchoe robusta* (KR) and *Kalanchoe blossfediana* (KB) were screened for their antibacterial activity against bacterial strains of *Clostridium perfringens, Enterobacter aerogenes, Salmonella typhimurium* and *Staphylococcus aureus*. Out of these three plants species, KR showed maximum zone of inhibition against *Clostridium perfringens* (12.6mm), KP shows maximum zone of inhibition against *Enterobacter aerogenes* (12.5mm), KR shows maximum zone of inhibition against *Salmonella typhimurium* (14mm) and KP shows maximum zone of inhibition against *Staphylococcus aureus* (12.3mm).

Phytochemical analysis of aqueous. acetonic and methanolic extracts were performed for qualitative analysis of flavonoids, tannins, phenols, saponins, cardiac glycosides, terpenoids, quinones, carbohydrates and alkaloids. The flavonoid content was found to be 620 µg/ml, 1120 µg/ml and 1250 µg/ml in KP, KR and KB respectively. The phenolic content was found to be 400 µg/ml, 800 µg/ml and 600 µg/ml in KP, KR and KB respectively.

Antioxidant activities are due to presence of various phytochemicals like flavonoids, isoflavones, flavones, anthocyanin, catechins and isocatechins. The antioxidant analysis of KP, KR and KB was performed for measuring % inhibition of plants extracts at different concentrations. It was observed that for both No assay and DPPH assay, % inhibition was significantly increasing with the increased dose of extract. Maximum % inhibition in DPPH assay was 70.7% while in NO assay it was 79.5%.

Keywords: Antibacterial, Medicinal plant, Phytochemicals, Antioxidant activity, Extracts

INTRODUCTION

Medicinal plants are the major source of ingredients for pharmaceutical and health care which are used worldwide. It is reported that approximately 70-80% of people worldwide trust predominantly on herbal or traditional medicines for their primary healthcare needs. Medicinal plants can have both synergic and preventive properties to cure the disease or prevent its appearance. Extracts obtained from medicinal plants have a crucial role for the production of semi-synthetic drugs. Active compounds produced during secondary metabolism play a vital role in biological properties called as secondary metabolites and are economically used in medicines, attars, pigments, insecticides and food additives. The biotechnological tools are significant to select, multiply, improve and analyse medicinal plants. The global demand for herbal medicine is rising than the mainstream industrialized pharmaceutical products [1].

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Shrubberies are always known to be a virtuous source of medicinal products that help to maintain human health. Investigation is already performed in recent times to acknowledge various medicinal products obtained from plants [2]. Plant extract is the major component which has lead to original medicinal product due to the availability of a vast variety of chemical constituents which plays vital role in drug discovery. Most pathogenic microorganisms show a lot of confrontation to antibiotics which has unfortunately shown side effects because of the drug material. Therefore, organically active compounds are considered as a priority, extracted from extracts of herbal medicinal plants [3]. At present, most of the scientists and researchers are focusing on developing the drugs and improve them against microbial infections by focusing their attention towards traditional medicines [4].

Therapeutic plants which were used traditionally are capable of producing a wide range of known therapeutic products [5]. Innovative antimicrobial drugs are developed using substances that are growth inhibitors or proficient in killing the pathogens and should be least harmful to the host cells. In recent times, an extensive research has been performed to witness antimicrobial, antihemolytic, antioxidant properties of medicinal plants [6]. Plants which are proficient in targeting sites, other than the target sites of the antibiotics possibly can be used against microbial pathogens which are drug resistant [7].

These medicinal plants were selected based on its uses in folk medicine. They were curtained for its antibacterial properties but were never precisely screened against pathogens or multidrug-resistant microorganism. The plant's extracts were also phytochemically analysed to acknowledge the phytoconstituents of the plants and the plants selected were *Kalanchoe pinnata, Kalanchoe robusta* and *Kalanchoe blossfediana.*
A defence mechanism is developed by a plant against the pathogen which constitutes of different chemical constituents and this reaction is pathogen specific as they were persuaded after the recognition of the pathogen. The first certification of scientific experimentation on the antibacterial properties of plant components was completed in 19th century. Latest microbiological techniques have proved that medicinal plants shows significant activities against human microbial and fungiform pathogens [8].

These plants are cultured as decorative houseplants and stalwart or succulent garden plants. They are popular because of their comfort of propagation, low water requirement, and wide variety of flower colors typically located in clusters particularly above the phylloclade. Bryophyllum a group of plant species of the family Crassulaceae and is included in the genus such as the "air-plant" Kalanchoe pinnata and contains bufadienolide cardiac glycosides [9].

A research performed on Sango bay area's medicinal plants located in southern Uganda (march- august 2004), by means of various organized meetings, surveys and member surveillance in desolated herbal plant collection areas. 186 herbal classes fitting to 163 genera and 58 families with therapeutic ethics were logged. Medicinal preparation was performed mainly as serums, syrups and brews which were accomplished in numerous ways. An appraisal of Ugandan and additional prose designated that 72 therapeutic plants described in this work have not been described earlier for having therapeutic value [10].

ISSN:0971-1260
Vol-22-Issue-16-August-2019

MATERIALS AND METHODS

Plant material collection

The plant material was collected from Punjab Agricultural University, Ludhiana.

Extract preparation

***Aqueous extract*:** Firstly, water was drawn out for extraction from the fresh stems and leaves of test plant. Plant parts (stems and leaves) were collected and washed thoroughly under running tap water for 3-5 times. Both plant parts were homogenized in 100ml of double distilled water followed by centrifugation at 1000 rpm with time taken as 5 minutes to discrete the the debris from liquid media. Supernatant collected, were then stored in falcon tubes below ambient temperature [11].

Methanol and Acetone extract: 5g of dried plant part (leaves and stems) of chosen *Kalanchoe* species was coarsely powdered and soaked separately in closed flasks carrying 100ml of methanol and acetone for 24 hours, and then shaken frequently for six hours and allowed to stand for 18 hours. The solution were filtered rapidly, following precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a twered flat-bottomed shallow dish, and dry at 105°C [11].

Antimicrobial assay

To check antimicrobial activity of test plants agar disc diffusion method was used. It is an experiment in which antibiotic-impregnated wafers were used to confirm whether bacteria gets exaggerated or affected by the use of antibiotics. During this trial, antibiotic discs were kept on an agar gelled plate where bacteria has been sited followed by its incubation for 24hrs at 34ºC-37ºC. When observed after 24 hours, if an antibiotic sojourns bacterium from growing, there would be a clear zone of inhibition around the disc. Size of zone of inhibition depends on how nominal the antibiotic is to inhibit the growth of bacterium because the stronger the antibiotic is, the larger is the zone of inhibition [12].

Whatman's No.1 filter paper was used for making discs. Filter paper was punched into 5mm of disc and then sterilized, each sterilized disc was incorporated with extracts. The material used in antimicrobial assay is stated in table no.1.

Antioxidant analysis

DPPH radical scavenging assay

DPPH (2,2- diphenyl-1-pcryl hydrazyl) acts as a stable and free radical which is purple in color, Its intensity is particularly measured at 510 nm spectrotometrically. [13] Antioxidant reduces DPPH to 2,3- diphenyl-1-pcyrl hydrazine and forms a colorless compound.

Nitric oxide scavenging assay

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Nitric oxide (NO) is generated from sodium nitroprusside which is measured by Griess reagent. At any physiological pH, sodium nitroprusside generates NO, which interact with oxygen to produce nitrite ions, followed by its estimation with Griess reagent. [14]

Phytochemical analysis

Parts of the *K. pinnata, K robusta, K. blossfeldiana* were subjected to preliminary phytochemical screening for the presence or absence of various primary and secondary metabolites.

Test for alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, aminoacids, tannins, saponins, terpenoids and quinones were done as per the protocol followed by Sandhu *et al* [15].

Estimation of Phytochemicals with the help of Spectrophotometer:
For the estimation of flavonoid and phenolic content, quercitin was taken as standard.

Estimation for the presence of Flavonoids
0.5ml solution of extract in methanol is separately mixed with 1.5ml methanol, 0.1 ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water and left at room temperature for 30 min.
The absorbance of the reaction mixture was measured at 415 nm spectrophotometrically.
[15].

RESULTS AND DISCUSSION

Antibacterial Testing

The plates were prepared on nutrient agar media and the discs soaked in the filtrate were kept on the plates. The plates were then kept in incubator for 24-48 hours at 37°C. The clear zone of inhibition formed around the disc confirms the antibacterial activity of the chemicals present in the respective plant extract taken.

The Fig.1 represents the antibacterial activity of *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* respectively against *Clostridium perfringens (MTCC NO. 450)*. Along X-axis are the names of plants species and along the Y-axis are the mean diameters (mm) of clear zones formed around the discs.

From Fig.1 it can be dogged that *aqueous extract* of *Kalanchoe robusta* shows maximum activity against *Clostridium perfringens,* whereas Acetonic extract of *Kalanchoe blossfeldiana* shows minimal activity. *Acetonic extract of Kalanchoe pinnata* shows a significant activity against *Clostridium perfringens* as the diameter of zone of inhibition is 13mm.

Methanolic extract of *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* shows significant antibacterial activity against *Clostridium perfringens (MTCC NO. 450).*

ISSN:0971-1260
Vol-22-Issue-16-August-2019

The Fig.2 represents the antibacterial activity of *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* respectively against *Enterobacter aerogenes* (MTCC NO. 7325). Along X-axis are the name of plants species and along the Y-axis are the mean diameters (mm) of clear zones formed around the discs.

From Fig.2 it can be dogged that aqueous extract of all the three plants shows maximum activity against *Enterobacter aerogenes* whereas acetonic extract *Kalanchoe pinnata* shows significant activity. Methanolic extract of *Kalanchoe blossfeldiana* shows a significant activity against *Enterobacter aerogenes* as the diameter of zone of inhibition is 11.3 mm.

Methanolic extract of three plants *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* shows significant antibacterial activity against *Enterobacter aerogenes* (MTCC NO. 7325*).*

The Fig.3 represents the antibacterial activity of three medicinal plants *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* respectively against *Salmonella typhimurium* (MTCC NO. 3231). Along X-axis are the name of plants species and along the Y-axis are the mean diameters (mm) of clear zones formed around the discs.

From Fig.3 it can be dogged that aqueous extract of all the three plants shows significant activity against *Salmonella typhimurium* whereas aqueous extract *Kalanchoe robusta* shows maximum activity. Acetonic extract of *Kalanchoe robusta* shows a significant activity against *Salmonella typhimurium* as the diameter of zone of inhibition is 14 mm.

Methanolic extract of *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* shows significant antibacterial activity against *Salmonella typhimurium* (MTCC NO. 3231).

The Fig.4 represents the antibacterial activity of three medicinal plants *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* respectively against *Staphylococcus aureus* (MTCC NO. 7405). Along X-axis are the name of plants species and along the Y-axis are the mean diameters (mm) of clear zones formed around the discs.

From Fig.3 it can be dogged that aqueous extract of all the three plants shows significant activity against *Staphylococcus aureus* (MTCC NO. 7405) whereas *Kalanchoe robusta's* methanolic extract shows minimum activity. Acetonic extract of *Kalanchoe robusta* shows a significant activity against *Staphylococcus aureus* (MTCC NO. 7405) as the diameter of zone of inhibition is 12 mm.

Acetonic extract of *Kalanchoe pinnata, Kalanchoe robusta and Kalanchoe blossfeldiana* shows significant antibacterial activity against *Staphylococcus aureus* (MTCC NO. 7405).

All the three extracts of *Kalanchoe blossfeldiana'* shows significant antibacterial activity against *Staphylococcus aureus* (MTCC NO. 7405).

Phytochemical tests:
Three plant species were studied based on their phytochemical properties. A phytochemical is a compound found in the plants that works with nutrients and dietary fiber to protect against disease. [16] They also help to slow down the process of aging.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

All three plant species *Kalanchoe pinnata*, *Kalanchoe robusta* and *Kalanchoe blossfeldiana* has been compared. The phytochemical testing on the plants was done for methanolic, aqueous and acetonic extracts.

Kalanchoe pinnata shows the maximum presence of phytochemicals among nine phytochemicals screened in the plant extracts, whereas *Kalanchoe robusta* shows less activity.

For aqueous as well as acetonic extracts of *Kalanchoe pinnata* and *Kalanchoe robusta* show the presence of number of phytochemical test and *Kalanchoe blossfediana* shows significant phytochemical activity among the nine phytochemical tested.

As the results obtained, it can be concluded that aqueous extract of three plants shows positive results in case of tannins, flavonoids, alkaloids, phenols and carbohydrates. It gave negative results in case of terpenoids and quinones which marks the absence of these components in the case of aqueous extract of *Kalanchoe pinnata*, *Kalanchoe robusta* and *Kalanchoe blossfediana*.

In case of acetonic extract, *Kalanchoe pinnata* and *Kalanchoe robusta* gave negative test for saponins while it is positive in case of *Kalanchoe blossfediana.* For presence of cardiac acid, ccetonic extract of *Kalanchoe robusta* gives positive test while it is absent in other two plants i.e. *Kalanchoe pinnata* and *Kalanchoe blossfediana*. Quinones are absent in acetonic extract of Kalanchoe pinnata while present in *Kalanchoe blossfediana* and *Kalanchoe robusta.*

Methanolic extract of three plants shows significant phytochemicals presence in all the three extracts i.e. acetonic, methanolic and aqueous extract. While, positive result is shown in all three plants in case of presence of alkaloids. The results are shown in table no. 2.

Flavonoids content estimation

Quercetin was taken as standard for the estimation of flavonoids. Absorbance was measured at 415nm at different concentrations of quercetin for preparing standard curve as shown in table 3.

Phenolic content estimation

For the estimation of phenolic content, quercetin was taken as standard. The absorbance in different concentration was taken at 765nm for preparing the standard curve as shown in table no. 4.

Phytochemical estimation analysis

Table 4 here represents the concentration of flavonoids in µg/ml in the three plants species. Along the X-axis is the name of plant species and along Y-axis is the concentration in ug/ml.

From table 4 it can be dogged that KB contains maximum amount of flavonoids (1250 µg/ml) among the three plants whereas KP contains the minimum amount (620 µg/ml). KR has significant amount of flavonoids that is 1120 µg/ml.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Table no. 5 here represents the concentration of flavonoids in µg/ml in the three plants species. Along the X-axis is the name of plant species and along Y-axis is the concentration in µg/ml.

From table no. 5 it can be dogged that KR contains maximum amount of flavonoids (800 µg/ml) among the three plants tested whereas KP contains the minimum amount (400 µg/ml). KB has significant amount of flavonoids that is 600 µg/ml.

Antioxidant activity

Nitric acid scavenging assay

Extract of three plants KP, KR and KB shows reduction in nitric oxide, as shown in the table no 6. Ascorbic acid was taken as the standard. The comparable scavenging effect of these three plants is shown in table 6.

Fig. 5 shows % inhibition of KP, KR and KB, out of which KB shows maximum inhibition i.e. 6.9% at 20μg/ml while KP showed minimum inhibition activity i.e. 1.16% at 20ug/ml concentration. KR showed significant inhibition activity at 20μg/ml. At 40μg/ml, it was observed that KB shows maximum antioxidant activity with % inhibition 17.9% while Kalanchoe robusta showed minimum inhibition activity i.e. 13.48%. While at 60μg/ml, KP shows maximum inhibition activity i.e. 43.4% as compared to KB that showed minimum activity (30.6%). At 80μg/ml, all three plants show significant inhibition activity. KR shows the maximum i.e. 66.9% while KP shows the minimum activity i.e. 60.6%. At 100μg/ml, KR shows maximum 83.03% of inhibition activity while KP shows 78.6% .

DDPH free radical scavenging

The reduction ability of the DPPH radial is determined by the decrease in its absorbance at 517nm induced by the antioxidants. [18] The scavenging effects of extracts of three plants increased with their concentrations. The percentage inhibition of Gallic acid was taken as standard. [19] Fig. 6 shows the percentage inhibition of three plants KP, KR and KB w.r.t standard i.e. Gallic acid.

Fig. 6 shows % inhibition of KP, KR and KB, out of which KP shows maximum inhibition i.e. 44.5% at 20μg/ml while KR shows minimum inhibition activity i.e. 13.3% at 20ug/ml concentration. KP shows maximum antioxidant activity both at 40 μg/ml and 60μg/ml as compared to other 2 plant species. At 80μg/ml, all three plants show significant inhibition activity. KP shows the maximum i.e. 62.4% while KR shows the minimum activity i.e. 48.4%. At 100μg/ml, KR shows 52.2% of inhibition activity, KP shows 70.7% and KR shows 61.9% of inhibition activity.

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ISSN:0971-1260
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ISSN:0971-1260
Vol-22-Issue-16-August-2019

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Strains used	**Antibiotics used**	**Media used**
Clostridium perfringens MTCC No.450	Penicillin	Nutrient agar media
Enterobacter aerogenes MTCC No.7325	Gentamicin	Nutrient agar media
Salmonella typhimurium MTCC No.3231	Streptomycin	Nutrient agar media and Tryptone soya agar media
Staphylococcus aureus MTCC No.7405	Streptomycin	Nutrient agar media

Table: 1 Materials used in antimicrobial assay

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Table: 2 Phytochemical analysis

	Kalanchoe pinnata			*Kalanchoe robusta*			*Kalanchoe blossfeldiana*		
	Acetone	**Aqueous**	**Methanol**	**Acetone**	**Aqueous**	**Methanol**	**Acetone**	**Aqueous**	**Methanol**
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	–	+	+	+	+
Saponins	–	+	–	–	+	–	+	–	+
Carbohydrate	+	+	+	–	+	+	–	–	+
Cardiac glycosides	–	+	–	+	+	+	–	+	–
Phenols	+	+	+	+	+	+	+	+	+
Quinones	–	+	+	+	–	–	+	–	–
Terpenoids	+	–	+	–	–	–	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+

+ = for the presence - = for the absence

Plant extract	**Concentration (µg/ml)**
Kalanchoe pinnata	620
Kalanchoe robusta	1120
Kalanchoe blossfeldiana	1250

Table: 3 Estimated flavonoid content of plants extracts

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Table no: 4 Estimated phenolic content of plant extracts

Plant extract	**Concentration (µg/ml)**
Kalanchoe pinnata	400
Kalanchoe robusta	800
Kalanchoe blossfeldiana	600

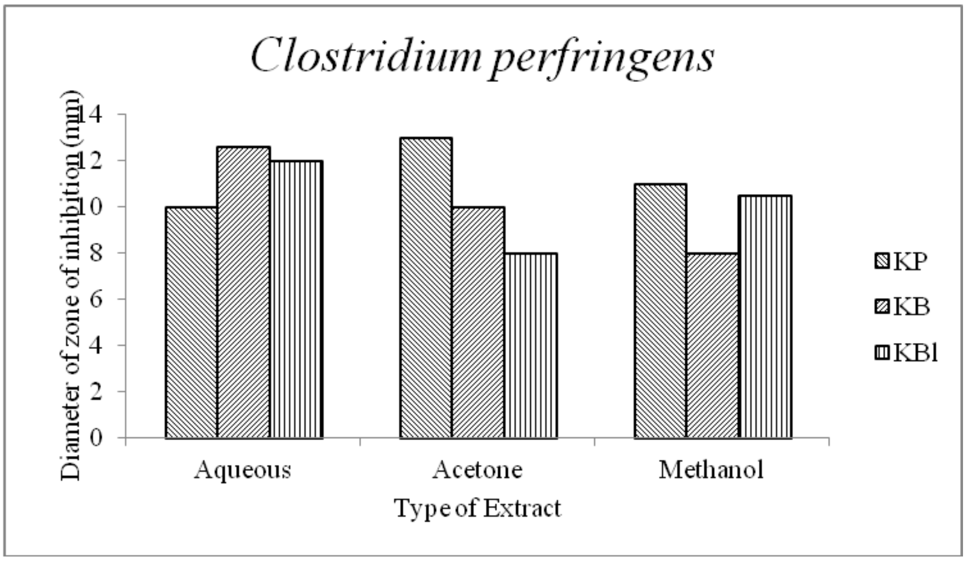


Fig.1 Zone of inhibition (mm) antibacterial activity of the *Kalanchoe pinnata, Kalanchoe robusta* and *Kalanchoe blossfediana* plants extract against *Clostridium perfringens* (MTCC NO. 450*)*.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

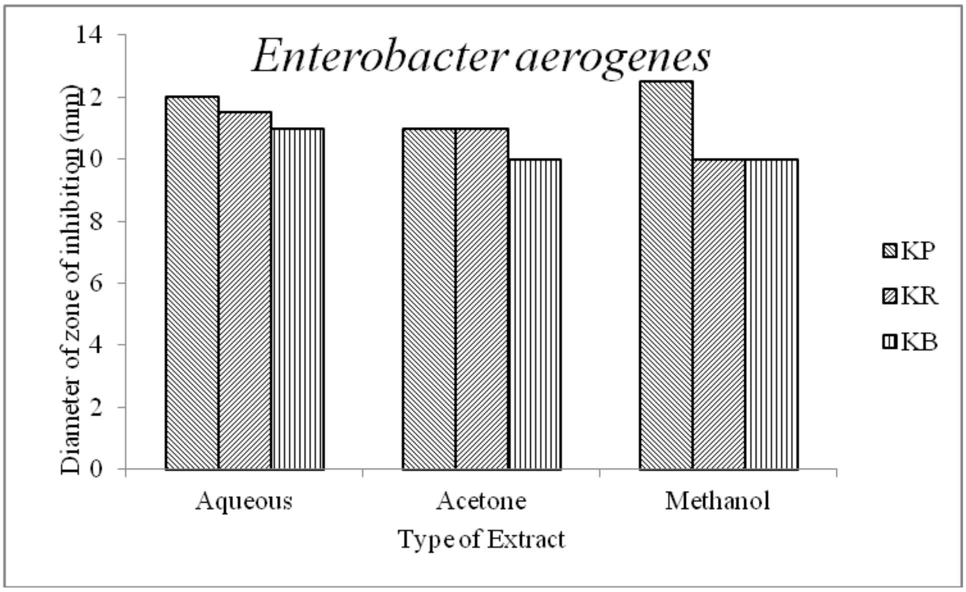


Fig.2 Zone of inhibition (mm) to show antibacterial activity of the *Kalanchoe pinnata, Kalanchoe robusta* and *Kalanchoe blossfediana* plants extract against *Enterobacter aerogenes* (MTCC NO. 7325*).*

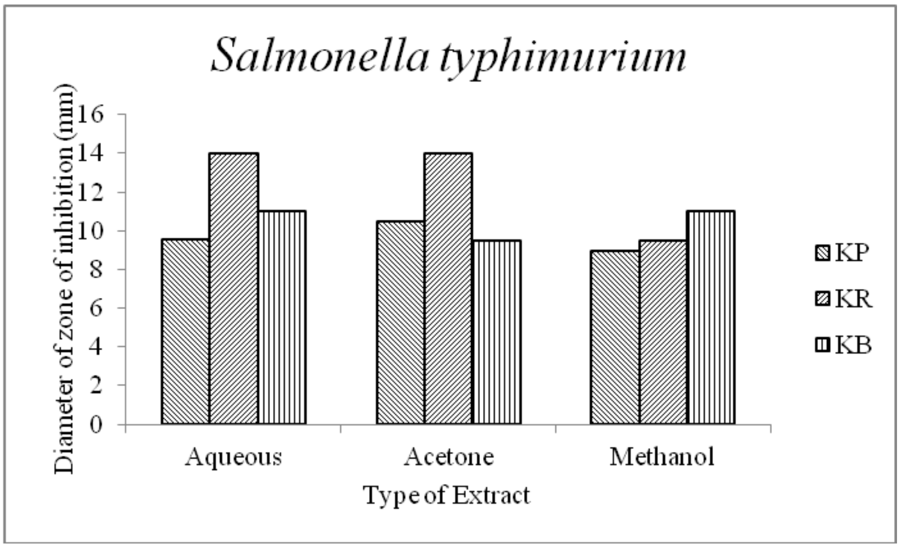


Fig.3 Zone of inhibition (mm) to show antibacterial activity of the *Kalanchoe pinnata, Kalanchoe robusta* and *Kalanchoe blossfediana* plants extract against *Salmonella typhimurium* (MTCC NO. 3231).

ISSN:0971-1260
Vol-22-Issue-16-August-2019

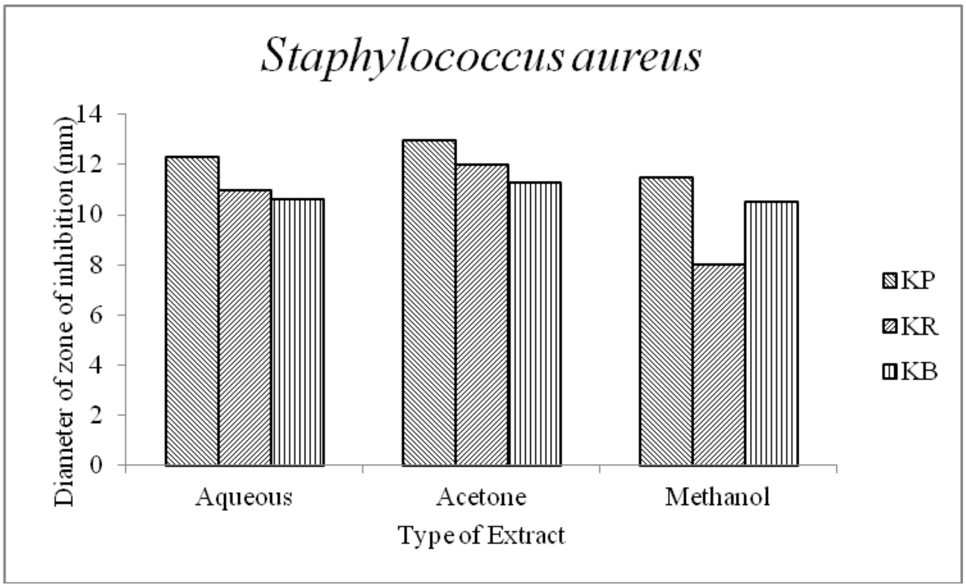


Fig.4 Zone of inhibition (mm) antibacterial activity of the *Kalanchoe pinnata, Kalanchoe robusta* and *Kalanchoe blossfediana* plants extract against *Staphylococcus aureus* (MTCC NO. 7405).

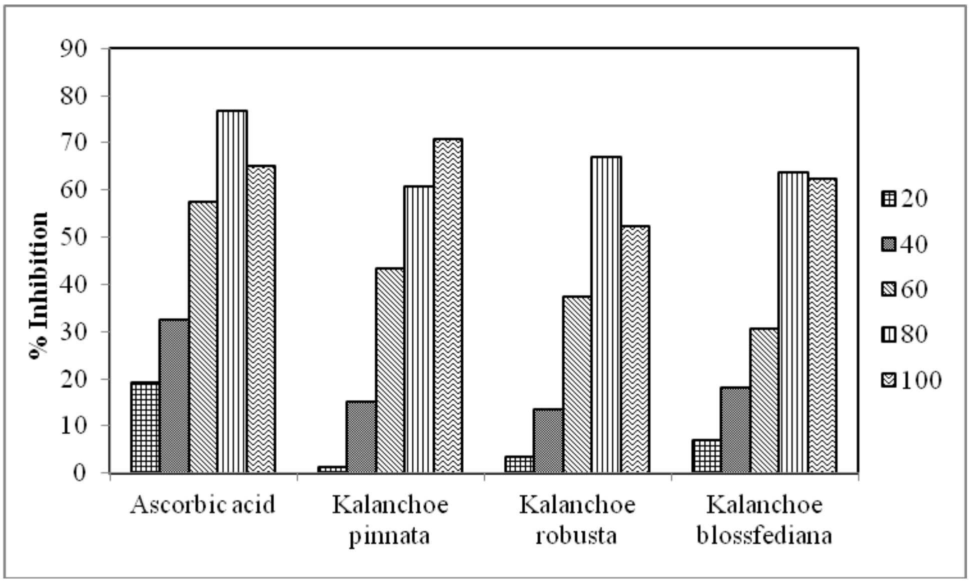


Fig. 5 Percentage inhibition of nitric oxide by three medicinal plants extracts.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

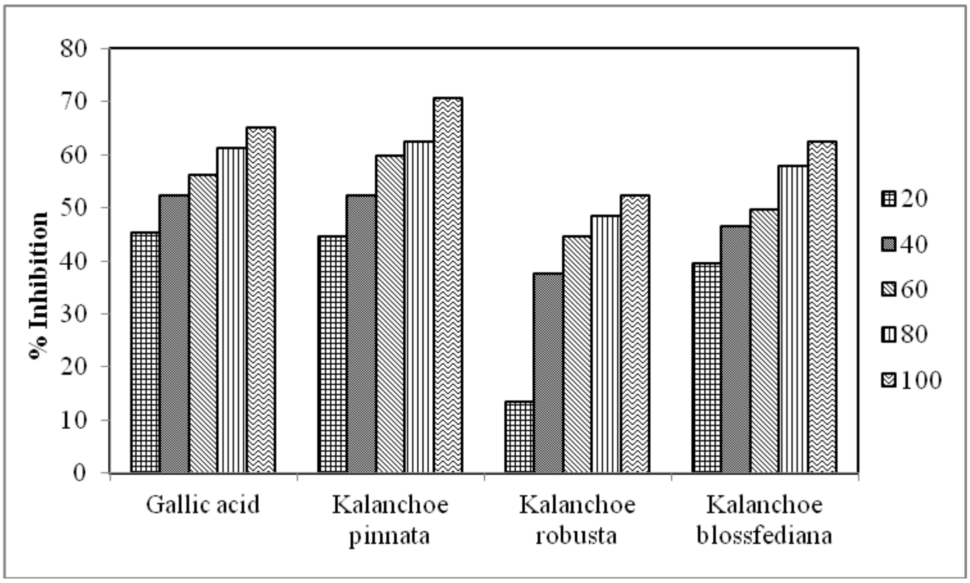
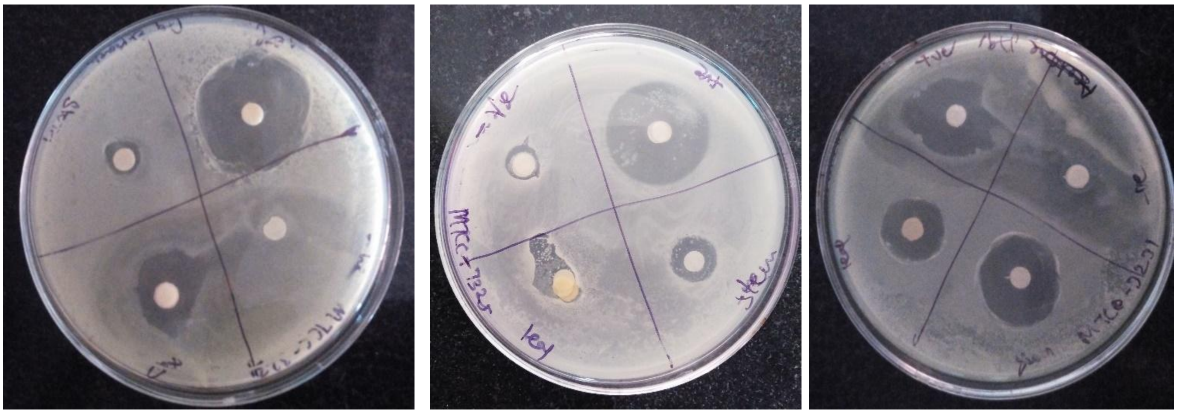


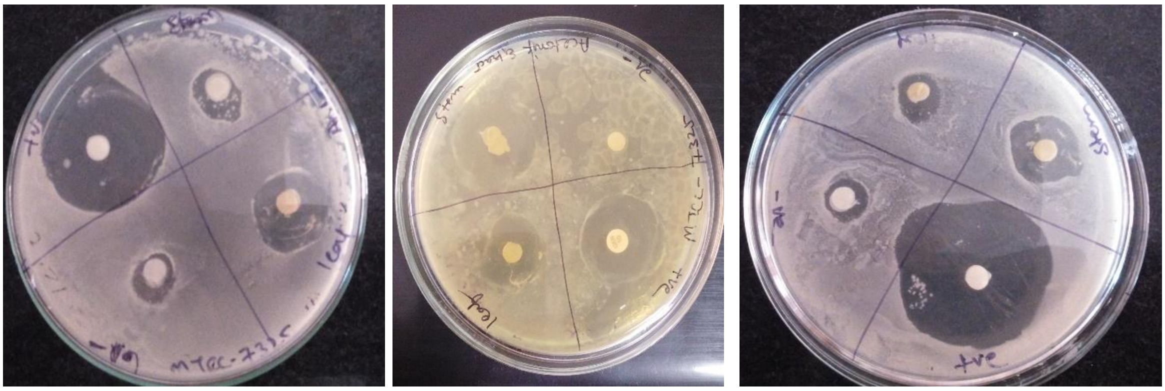
Fig. 6 Percentage inhibition by three medicinal plants extracts using DDPH scavenging assay.



(a) (b) (c)

Plate no.1: The antibacterial activity shown by (a) *Kalanchoe pinnata,* (b) *Kalanchoe robusta and* (c) *Kalanchoe blossfediana* extracts against for *Clostridium perfringens* (MTCC NO.450)

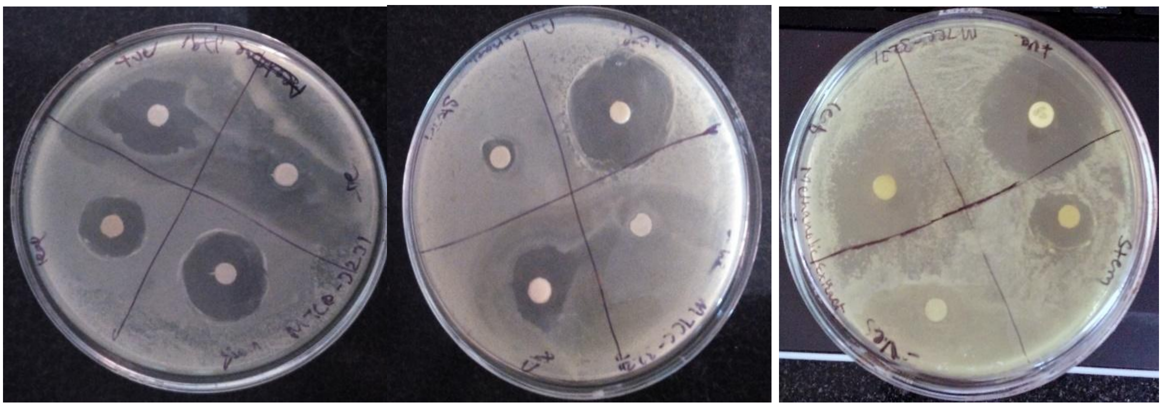
ISSN:0971-1260
Vol-22-Issue-16-August-2019



(a) **(b)** **(c)**

Plate no.2: The antibacterial activity shown by (a) *Kalanchoe pinnata,* (b) *Kalanchoe robusta and* (c) *Kalanchoe blossfediana* extracts against *Enterobacter aerogenes* (MTCC NO. 7325). **(b)** **(c)**

Plate no.3: The antibacterial activity shown by (a) *Kalanchoe pinnata,* (b) *Kalanchoe robusta and* (c) *Kalanchoe blossfediana* extracts against *Salmonella typhimurium* (MTCC NO. 3231)



(a) **(b)** **(c)**

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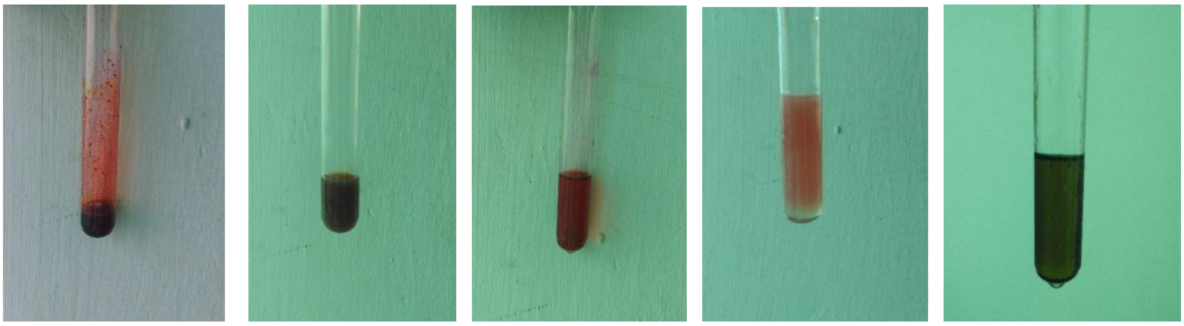
Plate no.4: The antibacterial activity shown by (a) *Kalanchoe pinnata*, (b) *Kalanchoe robusta and* (c) *Kalanchoe blossfediana* extracts against *Staphylococcus aureus* (MTCC NO. 7405)



(a) (b) (c) (d) (e)

Tube No.1: Positive phytochemical analysis of plant extracts (a) Alkaloids, (b) Phenols, (c) Cardiac glycosides, (d) Carbohydrates, (e) Saponi