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COMPARATIVE STUDY OF ANTIBACTERIAL PROPERTIES OF FLAVONOIDS OF LEAVES FROM DIFFERENT CACTUS, PERENNIAL GRASSES AND MEDICINAL PLANT

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ABSTRACT

Comparative study of antibacterial properties of flavonoids of leaves from different Cactus (Euphorbia caducifolia); perennial grasses (Panicum antidotale and Lasiurus sindicus) and medicinal plant (Anaegissus rotundifolia) against some important bacteria (G+ve or G-ve) was done. Flavonoid extracts of leaves from all the selected plants were found to possess strong antibacterial activity against these test pathogenic microbes, as revealed by ZOI=(mm± SD), AI, MIC=(mg/ml), MMC=(mg/ml) and TA=(ml/gm) of extracts against each sensitive test pathogenic bacteria were also evaluated. Most of the pathogens were found to be sensitive against these flavonoid extracts. Free flavonoid extract of grasses and medicinal plant showed higher activity against most of the pathogens. Euphorbia caducifolia is the most inactive plant in this study. All pathogens didn't show any activity against Euphorbia caducifolia except Proteus mirabilis which show 7.83±0.21 mm ZOI for free flavonoid. Highest ZOI (18.67±0.26) was recorded for free flavonoid of medicinal plant (Anaegissus rotundifolia) against Bacillus subtilis (G+ve bacteria) as well as other parameters like AI (1.245), MIC (0.039 mg/ml), MMC (0.78 mg/ml) were found higher against Bacillus subtilisand highest TA (1012.821 ml/gm) were found highest for free flavonoid of Lasiurus sindicus against Bacillus subtilis. The findings of the present study suggested the exploitation of flavonoid extracts of Anaegissus rotundifoliaand of Lasiurus sindicusfor future antibacterial drugs.

Key words: *Antibacterial, Flavonoids, Lasiurus sindicus, Anaegissus rotundifolia, Zone of Inhibition, Activity Index, Minimum Bactericidal Concentration and Total Activity.*

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INTRODUCTION:

Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity (1). Antibiotics were medical miracles during the Second World War but are now becoming impotent bacterial weaponry (2). This has caused an urgent need for the research of new and innovative ways to control bacterial invasions especially by multi-resistant pathogens such as *B. subtilis* (Gram +ve) and *P. aeruginosa* (Gram -ve) (3).

Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (4). Their activities are structure dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization. Recent interestin these substances has been stimulated by the potential health benefits arising from the antioxidant activities of the sepoly-phenolic compounds. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. These bioactive compounds exert antimicrobial activity through different

mechanisms. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (5).Phenolic compounds exhibit a considerable free radical scavenging (antioxidant) activity (6).The plants are vital source of innumerable number of antimicrobial compounds. Several phytochemical constituents like flavonoids (7), phenolics and polyphenols (8), tannins (9), etc. are effective antimicrobial substances against a wide range of microorganisms. One of the largest groups of chemical produced by plant is the alkaloids and their amazing effects on humans have led to the development of powerful painkiller medications (10).

Infectious diseases are the world's leading cause of premature deaths. In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world (11). Even though pharmaceutical companies produce number of new antibacterial drugs, but gradual resistance to these drugs has increased which is matter of global concern besides synthetic drugs are normally associated with side effects (hypersensitive, immune suppression etc). Use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. Present study is an effort towards this direction. Results reveal that all the tested extracts of selected plant exhibited growth inhibitory activity against one of the other bacterial strains selected.

Panicum antidotale and *Lasiurus sindicus* (perennial grasses) are more efficient at gathering Carbon dioxide and utilizing nitrogen from the atmosphere and recycled Nitrogen in the soil(12,13). Perennial grasses are more competitive under the conditions of high temperature, solar radiation and low moisture. These grasses have excellent soil binding capacity which helps to conserve soil in desert areas. These grasses are distributed in hotter and drier parts of India, Mediterranean region, tropical and southern Africa. This grass, fed green, turned into silage, or made into hay is said to increase flow of milk in cattle and impart a sleek and glossy appearance (14). Seeds of this grass are used as famine food by the tribal during severe conditions.

Klebsiella pneumonia more frequently causes lung destruction and pockets of pus in the lung (known asempyema), respiratory infections, such as bronchitis, which is usually a hospital-acquired infection (15). *Proteusmerabilis* cause obstruction and renal failure. It can also cause wound infections, septicemia and pneumonias, mostlyin hospitalized patients. *A. tumefaciens* (Plant pathogen) uses horizontal gene transfer to cause tumors "crown gall disease" in plants. It can be responsible for opportunistic infections in humans with weakened immune systems (16).*R.planticola* has been determined to cause severe pancreatitis in one case (17). *Bacillus subtilis* can contaminate food; however, they seldom result in food poisoning. *E. aerogens* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections.

MATERIALS AND METHODS:

Experimental design: Flavonoid extract of leaves of selected plants cactus (*Euphorbia caducifolia*); perennial grasses (*Panicum antidotale* and *Lasiurus sindicus*) and medicinal plant (*Anaegissus rotundifolia*)were prepared by hot extraction method (18) in Soxhlet assembly.

All the flavonoid extracts were then screened for evaluation of antibacterial activity by Disc Diffusion Assay (DDA) (19,20), in terms of Zone of Inhibition [ZOI=(mm± SD)], Activity Index (AI), Minimum inhibitory concentration [MIC=(mg/ml)], Minimum Bactericidal concentration [MBC=(mg/ml)] and Total activity [TA=(ml/gm)] of extracts against some medically important pathogenic microbes (*Bacillus subtilis=G+ve, Escherichia coli, Raoultella planticola, Enterobactor aerogens, Proteus mirabilis, Klebsiella pneumonia* and *Agrobacterium tumefaciens=G-ve* bacteria. The fraction showing best activity was then used for determining of minimum inhibitory concentration (MIC) by tube dilution method (21) and minimum bactericidal concentration (MBC).

Plant material: Leaves of selected plants *Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia* were collected in the month of August 2014 from the Central Arid Zone Research Institute (CAZRI), Jodhpur (Rajasthan). Plant samples were identified and deposited in the herbarium, Department of Botany, University of Rajasthan, Jaipur. Herbarium no. of these plants are recorded in table 1. The collected plant materials were transferred immediately to the laboratory cleaned with water (22) and selected plant parts were separately shade dried (23) until weight has been constant (24) (table-1).

Table 1: Selected plants:

S. No.	Name of plant	Herbarium No.
1.	*Euphorbia caducifolia*	RUBL211367
2.	*Panicum antidotale*	RUBL211364
3.	*Lasiurus sindicus*	RUBL211366
4.	*Anaegissus rotundifolia*	RUBL211363

Table 2: Flavonoid Extraction by hot extraction method from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*):

S. No.	Name of plant	Flavonoid	
		Free Flavonoid	Bound Flavonoid
1.	*Euphorbia caducifolia*	+	+
2.	*Panicum antidotale*	+	+
3.	*Lasiurus sindicus*	+	+
4.	*Anaegissus rotundifolia*	+	+

Preparation of plant extracts: The shade-dried leaves were powdered with the help of a grinder (25)and passed through 40mm meshes (26) and stored in clean container for further use (27).The dried powder material was extracted by hot extraction method (28) using the Soxhlet apparatus (29).

Determination of total Flavonoid Content: The total flavonoid content was determined according to the aluminum chloride colorimetric method (30). Rutin was chosen as a standard (the concentration range; 0.005 to 0.1mg mL^{-1}) and the total flavonoid content was expressed as milligram per gram of dry extracts.

Preliminary detection of flavonoids: Following methods were used to determine the presence of flavonoids in the plant samples:

Five 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract, followed by addition of concentrated H_2SO_4. A yellow color observed in each extract indicated the presence of flavonoids. The yellow color disappeared on standing.

Ethyl acetate test: A portion of the powdered plant sample was heated with10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and4ml of the filtrate was shaken with 1ml of dilute ammonium solution. A yellow coloration was observed indicating a positive test for flavonoids.

Extraction of flavonoids: Collected plant leaves were separately shade dried, finely powered using a blender and subjected to extraction following the method of Subramanian and Nagarjan (29). About 100 g of each finely powered sample was Soxhlet extracted with 80% hot methanol (500 ml) on a water bath for 24 h and filtered. Each filtrate was re-extracted successively with petroleum ether (fraction I), ethyl ether(fraction II) and ethyl acetate (fraction III) using separating funnel. Petroleum ether fractionswere discarded as being rich in fatty substances, whereas ethyl ether and ethylacetate fractions were analysed for free and bound flavonoids, respectively. Ethyl acetate fraction of each of the samples was hydrolysed by refluxing with 7% H_2SO_4 for2 h (for removal of bounded sugars from the flavonoids) and filtered. The filtrate was extracted in ethyl acetate and washed with distilled water to neutrality. Ethyl ether (free flavonoid) and ethyl acetate fractions (bound flavonoids) thus obtained were dried in vacuo and weighed. The extracts were stored at 4°C and were re-suspended in their respective solvents to get 10 mg/ml concentration for antimicrobial assay.

Flavonoid extraction: Leaves of selected plants were taken for flavonoids extraction following the well-established method (29). Each extract was dried in vacuo and storedat 4 °C in airtight vials for further use. Percent (%) extractive values were calculated by the following formula-

$$\text{Percent (\%) Extracts} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Drugs and Chemicals Used:

Drugs: Drugs: Streptomycin, Ciprofloxacin, Ceftriaxone (Standard Antibiotics for bacteria)

Chemicals: Sabouraud Dextrose Agar Medium (SDA) (Peptone 10g;Dextrose 20g; Agar 20g in 1000ml of distilled water; pH adjusted to 6.8-7.0 at 27±2°C), Ammonium chloride, ammonium solution, Ethyl acetate, H_2SO_4.

Micro-organisms: The organisms used in this study were seven bacteria (sixG-ve andone G+ve). Selected bacteria were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on Muller-Hinton Agar Medium (MHA) at 37±2°C and sub cultured regularly (after every 30 days) and stored at 4^oC as well as at $–80^oC$ by preparing suspensions in 10%glycerol (31) (table-3).

Table 3: Selected pathogens:

S. No.	Pathogens Type	Name of Pathogens	G+ve/ G-ve	Specimen no.
1.	**Bacteria**	*Escherichia coli*	G-ve	MTCC-46
2.		*Raoultella planticola*	G-ve	MTCC-530
3.		*Bacillus subtilis*	G+ve	MTCC-121
4.		*Enterobactor aerogens*	G-ve	MTCC-111
5.		*Proteus mirabilis*	G-ve	MTCC-3310
6.		*Klebsiella pneumoniae*	G-ve	MTCC-4030
7.		*Agrobacterium tumefaciens*	G-ve	MTCC-431

Preparation of Test Pathogens and Disc Diffusion Assay (DDA): Initial screening of different extracts for their antibacterial activity carried out using MHA and NA media but, did not reveal any significant difference, thus further studies were carried out using NA medium only (32). Bacterial strains were grown and maintained on NA medium. Bacterial growth can observe after a minimum of 18 hours and occasionally until 24 hours. Disc diffusion assay (DDA) was performed for screening. NA base plates were seeded with bacteria

(inoculum size 1×10^8 CFU/ml for bacteria). Sterile filters paper discs (Whatman no. 1, 5mm in diameter) were impregnated with 100 µl of each of the extracts (100 mg/ml) to give a final concentration of 1 mg/disc and left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination (33). Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum (34,35). Extract discs were then placed on the preseeded agar plates. Each extract was tested with Streptomycin (10mcg/disc), Ciprofloxacin (10mcg/disc) and Ceftriaxone (10mcg/disc) commercial discs of antibiotics as positive control (standard) and experiment was done thrice (36) for bacteria. The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 37°C for bacteria (24 h) and 27°C (37). Zone of Inhibition (ZOI) or depressed growth of microorganisms was measured. This method was followed by various researchers (38,39).

Zones of Inhibition (ZOI): Zones of Inhibition [ZOI=**(mm± SD)]**, measured in mm (mean value; include 5 mm diameter of disc), indicate that no bacterial growth around the tested flavonoid extract disc. ZOI measured and compared with the standard reference antibiotics (40-42).

Activity index (AI): Activity index foreach extract was calculated by the formula(43).

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

Determination of Minimum Inhibitory Concentration (MIC) by Serial Dilution Method/ Micro Broth Dilution (MBD) Method: Minimum inhibitory concentrations (MICs) are considered as the "gold standard" for determining the susceptibility of the organisms to antimicrobials. MIC was determined for plant extracts those showingantimicrobial activities against test pathogens in disc diffusion assay (44). Broth micro-dilution method (45) was followed for determination of MIC values. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms)to make 10mg/ml final concentration and then was added to test tubes containing 1 ml of sterile NA media. The tubes were then inoculated with a drop of microbial suspension (for bacteria 1×10^8 CFU/ml) and the tubes were incubated at 37±2°C for 24hrsin a BOD (Biological Oxygen Demand) incubator. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity (46) in the wells of microtiter plate. A tube containing nutrient broth without extract was taken as control. The least extra concentration which inhibited the growth of the test organisms was taken as MIC (47). Broth media of 96-wells of microtiter plates using two serial dilutions. There after 100µl inoculum of standard size was added to each well. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of bacteria.

The MIC values were taken as the lowest concentration of the extracts in the test tubes that showed no turbidity after incubation (48). The turbidity of the test tube was interpreted as visible growth of microorganisms.

Determination of Minimum bactericidal concentration (MBC) by Serial Dilution Method/ Micro Broth Dilution (MBD) method: It is defined as the concentration of the antimicrobial that results in a 99.9% reduction in CFU/ml for bacteria, compared with the organism concentration in the original inoculum (49). Equal volume of various concentration of each extract and nutrient agar were mixed in micro-tubes to make up 0.5ml of solution. Then 0.5ml of McFarland standard of the organism suspension was added to each tube (50). The tubes were incubated aerobically at 37 ^{o}C for 24 h for bacteria in a BOD incubator and observed for change in turbidity after 24 hrs and 48 hrs comparisons with the growth and sterility controls. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on Nutrient Agar followed by incubation. The MBC was determined by sub-culturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on sub-culturing was taken as MBC (51). MBC was calculated for those extracts that had shown high antimicrobial activity against tested organisms.

Total activity (TA-mg/ml): Total activity is the volume at which test extract can be diluted without loosing the ability to kill microorganisms (52). It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/gm. In mathematical terms it can be expressed as:

$$\text{Total activity (TA)} = \frac{\text{Amount extracted from 1g plant material}}{\text{MIC of the extract}}$$

Statistical Analysis: Mean value and standard deviation were calculated for each test pathogens. Data were analyzed by one-way ANOVA and p values ($p > 0.005$) were considered significant (53).

RESULTS:

Preliminary phyto–profiling: The preliminary phyto–profiling of flavonoid extraction from leaves of selected plants were carried out (table 3) according to Farnsworth (54). Free flavonoid show higher yield as compare to bound flavonoidof all the selected plants. The highest yield (mg/gm w/w in dry weight) was recorded (table 4) for free flavonoid of *L. sindicus* (39.5)followed by *P. antidotale* (34.52).

Table 4: Preliminary phyto-profile of Flavonoid Extraction (Free Flavonoid and Bound Flavonoid) from leaves of selected plants:

S. No.	Phytochemical estimation	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**
1.	%Yield	1.86	1.78	3.45	2.94	3.95	3.29	1.84	1.70
2.	Yield in mg/gm DW	18.6	17.8	34.52	29.38	39.5	32.9	18.43	6.143
3.	Yield in gm (30gm)	6.200	5.933	11.507	9.793	13.167	10.967	16.96	5.653
4.	Colour	Green	Green	Dark Green	Greenish Yellow	Brown	Greenish Yellow	Dark green	Green

Antibacterial activity: Extraction of flavonoid from leaves of selected plants were found to possess strong antibacterial activity against these test pathogens, in terms of Zone of Inhibition [ZOI=(mm± SD)] and Activity Index (AI), Minimum inhibitory concentration [MIC=(mg/ml)], Minimum bactericidal concentration [MBC=(mg/ml)] and Total activity [TA=(ml/gm)] of extracts against each sensitive test pathogens were also evaluated.

Zone of Inhibition [ZOI=(mm± SD)]:

Antibacterial activity: Most susceptible organism in this study was *B. subtilis* (G+ve bacteria) against which, most of the plant extracts showed higher zone of inhibition as compare to the other organisms (G-ve bacteria). Free flavonoid show higher activity as compare to bound flavonoid of all the selected plants. Highest ZOI were recorded for leaves of *Anaegissus rotundifolia* (18.67±0.26 mm) followed by *Lasiurus sindicus* (18.50±0.64 mm)against *B. subtilis* (G+ve bacteria) (table-5).

Table 5: Zone of Inhibition (ZOI) (mm± SD) of Flavonoid extraction from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*) against tested pathogens(G+ve and G-ve bacteria):

S. No.	Name of Pathogen	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	**Bound**	**Free**	**Bound**	**Free**	**Bound**	**Free**	**Bound**
1.	*Escherichia coli*	-	-	16.67± 0.21	-	18.33± 0.24	-	16.50± 0.27	15.17± 0.67
2.	*Raoultella planticola*	-	-	8.50± 0.21	7.33± 0.26	9.33± 0.26	8.50± 0.64	12.33± 0.24	10.83± 0.26
3.	*Bacillus subtilis*	-	-	15.33± 0.26	-	18.50± 0.64	-	18.67± 0.26	16.83± 0.64
4.	*Enterobactor aerogens*	-	-	7.83± 0.26	7.33± 0.21	8.67± 0.24	7.33± 0.26	10.67± 0.24	9.33± 0.26
5.	*Proteus mirabilis*	7.83± 0.21	-	9.83± 0.26	7.50± 0.24	11.83± 0.24	8.67± 0.21	11.33± 0.24	9.50± 0.26
6.	*Klebsiella pneumoniae*	-	-	9.83± 0.26	7.33± 0.21	11.50± 0.64	7.33± 0.24	14.50± 0.21	11.67± 0.27
7.	*Agrobacterium tumefaciens*	-	-	8.67± 0.24	7.50± 0.26	9.83± 0.21	8.67± 0.24	12.33± 0.27	10.50± 0.24

Activity Index (AI): Most susceptible organism in this study was *B. subtilis* (G+ve bacteria) against which, most of the plant extracts showed higher activity as compare to the other organisms (G-ve bacteria). Free flavonoid show higher activity as compare to bound flavonoid of all the selected plants. Highest AIvalues were recorded for *A. rotundifolia* (1.245) followed by*L. sindicus* (1.233) against *B. subtilis* (table-6).

Table 6: Activity index (AI)of Flavonoid extraction from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*) against tested pathogens(G-ve and G-ve bacteria):

S. No.	Name of Pathogens	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound
1.	*E. coli*	--	--	1.042	--	1.146	--	1.031	0.948
2.	*R. planticola*	--	--	0.567	0.489	0.622	0.567	0.822	0.722
3.	*B. subtilis*	--	--	1.022	--	1.233	--	1.245	1.122
4.	*E. aerogens*	--	--	0.489	0.458	0.542	0.458	0.667	0.583
5.	*P. mirabilis*	0.489	--	0.614	0.469	0.739	0.542	0.708	0.794
6.	*K. pneumoniae*	--	--	0.655	0.489	0.767	0.489	0.967	0.778
7.	*A. tumefaciens*	--	--	0.578	0.500	0.655	0.578	0.822	0.700

Minimum inhibitory concentration [MIC=(mg/ml)]: MICs were evaluated for those plant parts extracts, which had shown activity in 'Disc Diffusion assay'. The range of MIC of Alkaloid extracts recorded was 0.020 - 10 mg/ml. In the present investigation lowest MIC values (0.039 mg/ml) were recorded for free flavonoid extracts of *Anaegissus rotundifolia*and *Lasiurus sindicus* followed bybound flavonoid of*Anaegissus rotundifolia*(0.078 mg/ml) against *B. subtilis*(table 7).

Table 7: Minimum Inhibitory Concentration (MIC-mg/ml)of Flavonoid extraction from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*) against tested pathogens(G-ve and G-ve bacteria):

S. No.	Name of Pathogens	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound
1.	*E. coli*	--	--	0.156	--	0.078	--	0.156	0.313
2.	*R. planticola*	--	--	2.500	5.000	2.500	2.500	2.500	1.250
3.	*B. subtilis*	--	--	0.156	--	0.039	--	0.039	0.078
4.	*E. aerogens*	--	--	5.000	5.000	5.000	5.000	1.250	2.500
5.	*P. mirabilis*	5.000	--	2.500	5.000	1.250	5.000	1.250	2.500
6.	*K. pneumoniae*	--	--	1.250	5.000	2.500	5.000	0.156	2.500
7.	*A. tumefaciens*	--	--	2.500	5.000	1.250	2.500	2.500	1.250

Minimum bactericidal Concentration (MBC = mg/ml): The highest dilution that yielded no single pathogen was taken as the Minimum microcidal concentration. The range of MBC of flavonoid extracts recorded was 0.020 - 10 mg/ml. In the present investigation lowest MIC values (0.078mg/ml) were recorded for free flavonoid extracts of *Anaegissus rotundifolia*and *Lasiurus sindicus* followed bybound flavonoid of *Anaegissus rotundifolia*(0.156 mg/ml) against *B. subtilis* (table 8).

Table 8: Minimum Bactericidal Concentration (MBC-mg/ml)of Flavonoid extraction from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*) against tested pathogens(G-ve and G-ve bacteria):

S. No.	Name of Pathogens	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound
1.	*E. coli*	--	--	0.313	--	0.156	--	0.313	0.625
2.	*R. planticola*	--	--	2.500	5.000	2.500	5.000	5.000	1.250
3.	*B. subtilis*	--	--	0.313	--	0.078	--	0.078	0.156
4.	*E. aerogens*	--	--	10.000	10.000	10.000	10.000	2.500	5.000
5.	*P. mirabilis*	5.000	--	5.000	5.000	2.500	10.000	2.500	5.000
6.	*K. pneumoniae*	--	--	2.500	5.000	5.000	10.000	0.313	5.000
7.	*A. tumefaciens*	--	--	5.000	10.000	2.500	2.500	5.000	5.000

Total activity [TA=(ml/gm)]: Total activity indicated the volume upto which the extract could be diluted without losing the ability to kill the microorganisms. In the present investigation higher TA values were recorded for free flavonoids as compare to the bound flavonoids. Highest TA value was observed (1012.821 ml/gm) byfree flavonoid extractagainst *Bacillus subtilis*followed by bound flavonoid extract(506.410 ml/gm) against *Escherichiacoli*of *Lasiurus sindicus*(table 9). It indicates that *Lasiurus sindicus* has higher activity as compare to other selected plants.

Table 9: Total Activity (TA-ml/gm)of Flavonoid extraction from leaves of selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*) against tested pathogens(G-ve and G-ve bacteria):

S. No.	Name of Pathogens	*Euphorbia caducifolia*		*Panicum antidotale*		*Lasiurus sindicus*		*Anaegissus rotundifolia*	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound
1.	*E. coli*	--	--	110.288	--	506.410	--	118.141	54.185
2.	*R. planticola*	--	--	6.904	11.752	15.800	13.160	7.372	13.568
3.	*B. subtilis*	--	--	221.282	--	1012.821	--	472.564	217.436
4.	*E. aerogens*	--	--	3.452	2.938	7.900	6.580	14.744	6.784
5.	*P. mirabilis*	3.72	--	6.904	5.876	31.600	6.580	14.744	6.784
6.	*K. pneumoniae*	--	--	6.904	11.752	15.800	6.580	118.141	6.784
7.	*A. tumefaciens*	--	--	6.904	23.504	31.600	13.160	7.372	13.568

DISCUSSION:

Most susceptible organisms observed in the investigations are *B. subtilis*and *Escherichia coli* against which, all the plant extracts showed good antibacterial activities. Highest antibacterial activity recorded by *Lasiurus sindicus* against *B. subtilis* (G+ve bacteria).

Phytochemical constituents such as tannins, flavonoids, steroids and several other aromatic compounds of plant that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. This may therefore explain the demonstration of antibacterial activity by the plant extracts. The demonstration of the antibacterial activity against bacteria may be indicative of the presence of broad spectrum antibiotic compounds. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times.

G+ve bacteria *B. subtilis* was the most susceptible organism as compare to the G-ve bacteria (*E. coli, R. planticola, E. aerogens, P. mirabilis, K. pneumonia* and *A. tumefaciens*) which supported the finding that plant extracts are usually more active against G+ve bacteria than G-ve bacteria (2,3,25,55-74).Susceptibility differences between G+ve and G-ve bacteria may be due to cell wall structural differences between these classes of bacteria. In the G-ve bacterial cell wall outer membrane appears to act as a barrier to many substances including synthetic and natural antibiotics (72).

Extracts under this study not only inhibit the bacterial growth but the ZOI developed, was more or less permanent when compared with the ZOI developed by

the standard drug used, as after sometime bacterial colonies could be easily seen in ZOI developed by standard drugs. In the light of the fact that micro-organism are becoming resistant against the drugs in use, present investigation is of great significance, as far as the future drugs are concerned and the selected plants could be used by the pharmaceutical industries for preparing plant based antimicrobials drugs. Perennial grasses (*P. antidotale* and *L. sindicus*) and *A. rotundifolia* easily grow in harsh climatic conditions or xeric conditions and require less care; hence its use as raw material for preparing drugs would definitely be economical.

Screening of all the selected plants (*Euphorbia caducifolia, Panicum antidotale, Lasiurus sindicus* and *Anaegissus rotundifolia*)under investigation so far has not been worked out for flavonoids. Mostly crude extracts have been screened and that too without MIC, MBC and TA determination. Such studies could only indicate their antibacterial potential but are not helpful in establishing them as an alternative forantibiotic. In the present study IZ, AI, MIC, MBC and TA have been evaluated for each extract. For most of the extracts MIC values recorded were very low, indicating strong bio-efficacy of the plant. In an overview of the bioactivity data obtained from the current investigation, it can be highlighted that the tested extracts have great potential to inhibit bacteria. There is a need for further investigation to explore the promising antibacterial properties of the selected plants.

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