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RESEARCH ARTICLE

Identification of Antibacterial Efficacy of Flavonoids of *Anaegissus rotundifolia* Against Some Pathogens

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ABSTRACT

Introduction: Antibacterial efficacy of flavonoids (bound and free) of different parts (root, stem, bark, flower, and gum) of Anaegissus rotundifolia was evaluated against seven bacteria (Bacillus subtilis = Gram-positive, Escherichia coli, Raoultella planticola, Enterobactor aerogens, Proteus mirabilis, Klebsiella pneumoniae, and Agrobacterium tumefaciens = Gram-negative bacteria). **Material and Method:** Flavonoids extracts of all the plant parts were found to possess strong antibacterial efficacy against these test pathogens, as revealed by zone of inhibition (ZOI [mm ± standard deviation]), activity index (AI), minimum inhibitory concentration (MIC [mg/ml]), minimum bactericidal concentration (MBC [mg/ml]), and total activity (TA [ml/g]) of extracts against each sensitive test pathogens were also evaluated. All the pathogens were found to be sensitive against these flavonoid extracts. **Result:** Flavonoid extract of the root and stem showed the best activity against B. subtilis (Gram-positive bacteria), ZOI (16.50 ± 0.24 mm), AI (1.100), MIC (0.078 mg/ml), and MBC (0.157 mg/ml). Highest TA (456.410 ml/g) of gum alkaloid was found against B. subtilis (G +ve bacteria). The findings of the present study suggested the exploitation of alkaloid extracts of Anaegissus rotundifolia for future antimicrobial drugs.

Keywords: Activity index, *Anaegissus rotundifolia,* antibacterial, flavonoids, minimum bactericidal concentration, minimum inhibitory concentration, total activity, zone of inhibition

INTRODUCTION

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.[1] Antibiotics were medical miracles during the Second World War but are now becoming impotent bacterial weaponry. This has caused an urgent need for the Res of new and innovative ways to control bacterial invasions especially by multi-resistant pathogens such as *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative).[2] 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.[3]

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Even though pharma companies produce a 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. The present study is an effort toward this direction. Results reveal that all the tested extracts of selected plant exhibited growth inhibitory activity against one of the other bacterial strains selected. The essential oils and the extracts of many plants have been prepared and screened for their antimicrobial and antioxidant activities.[4,5] Flavonoids are known to be synthesized by plants in response to microbial infection; thus, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Flavonoid-rich plant extracts from different species have been reported to possess antibacterial activity.[6,7]

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, anti-apoptosis, anti-aging, and improvement of endothelial function, as well as inhibition of angiogenesis, and cell proliferation activities.[8] Non-toxic glycoside can also be hydrolyzed to release phenolic compounds that are toxic to microbial pathogens. These bioactive compounds exert antimicrobial activity through different mechanisms. Phenolic compounds exhibit a considerable free radical scavenging (antioxidant) activity.[9] The plants are a vital source of innumerable number of antimicrobial compounds. Several phytochemical constituents such as flavonoids,[10] phenolics, and polyphenols,[11] and tannins[12] are effective antimicrobial substances against a wide range of microorganisms. One of the largest groups of chemical produced by the plant is the alkaloids, and their amazing effects on humans have led to the development of powerful painkiller medications.[13]

Klebsiella pneumonia more frequently causes lung destruction and pockets of pus in the lung (known as empyema), respiratory infections, such as bronchitis, which is usually a hospital-acquired infection.[14] *Proteus mirabilis* causes obstruction and renal failure. It can also cause wound infections, septicemia, and pneumonia, mostly in hospitalized patients. *Agrobacterium 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.[15] Major causative agent of nosocomial infections is *Enterobacter aerogenes* along with *Escherichia coli. Raoultella planticola* has been determined to cause severe pancreatitis in one case.[16]

Anaegissus rotundifolia is a medium-sized tree, about 6 m long. Young parts (branches, leaves, and inflorescence) cinereo-tomentose. Leaves alternate, many younger ones elliptic or suborbiculate, the mature ones orbiculate or suborbiculate, slightly broader than long, up to 2 cm in diameter, apex obtuse or emarginated, generally mucronate, silvery pubescent, petioles up to 3 mm long. Gum ghatti, as its gum is known is an exclusive product of India and Sri Lanka. Total gum ghatti production is 900–1150 m/ year and over 80% of it is exported to USA and Europe.[17] In this plant low dose (2 ml/plant) is suggested as higher doses have resulted in bark detachment which causes the plant mortality after 3–4 years.

Edible Uses

The gum that exudes from the trunk, known as "ghatti gum," has been used in sweetmeats and as an emulsifier in the food industry.

Medicinal Uses

The plant is used in treating snake bites and scorpion stings in India.

Agroforestry Uses

The tree is a good survivor on eroded land. It is used in riverbank stabilization. The tree contributes to soil nutrient cycling, exhibiting high leaf litter decomposition rates.

Other Uses

Ghatti gum is an exudation obtained from the wood. Composed of yellowish-white tears, it is soluble in water, giving very sticky mucilage. It is a good substitute for gum arabic and is used in calico printing, for sweetmeats, in dye processes, and as a binding agent in Pharms. The leaves and bark are used for tanning. The leaves yield a black dye that is used commercially in India. Produces a heavy hardwood with a density of 760940 kg/cu m. Shrinkage on seasoning is moderate to high, and the wood is difficult to season as it is liable to warping, splitting, and surface checking. Shrinkage on seasoning is moderate to high, and the wood is difficult to season as it is liable to warping, splitting, and surface checking. It is possible to modify surface checking completely by soaking in solutions of 50% polyethylene glycol 600 for 1 day. The wood is hard, strong and can be difficult to saw when mixed with other woods can make good packing and writing paper. The wood is used for erecting fences on field bunds. It yields good charcoal and firewood with an energy value of 17600–20500 kJ/kg.

MATERIALS AND METHODS

Experimental design

Flavonoid extracts of different parts (root, stem, bark, flower, and gum) of selected plant *A. rotundifolia* were prepared by hot extraction method[18] in Soxhlet assembly.

All the flavonoid extracts were then screened for evaluation of antimicrobial activity by disc diffusion assay (DDA),[19,20] in terms of zone of inhibition (ZOI [mm ± standard deviation (SD)]), activity index (AI), minimum inhibitory concentration (MIC [mg/ml]), minimum microcidal (bactericidal/fungicidal) concentration (mg/ml), and total activity (TA [ml/g]) of extracts against some medically important pathogenic microbes (*B. subtilis* = Gram-positive, *E. coli, R. planticola, E. aerogenes, P. mirabilis, K. pneumoniae,* and *A. tumefaciens* = Gram-negative bacteria). The fraction showing best activity was then used for determining of MIC by tube dilution method[21,22] and minimum bactericidal concentration (MBC).

Plant material

Different parts (root, stem, bark, flower, and gum) of selected plant *A. rotundifolia* (RUBL211363) were collected in the month of August 2014 from the Central Arid Zone Res 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 was recorded in Table 1. The collected plant materials were transferred immediately to the laboratory cleaned with water[23] and selected plant parts were separately shade dried[24] until weight has been constant.[25]

Preparation of plant extracts

The shade-dried plant parts were powdered with the help of a grinder[26] and passed through 40 mm meshes[27] and stored in a clean container for further use.[28] The dried powder material was extracted by hot extraction method[29] using the Soxhlet apparatus.[30]

Determination of total flavonoid content

The total flavonoid content was determined according to the aluminum chloride colorimetric method.[31] Rutin was chosen as a standard (the concentration range; 0.005–0.1 mg/mL), 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:

1. Five 5 ml 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.
2. Ethyl acetate test: A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonium solution. A yellow coloration was observed indicating a positive test for flavonoids.

Extraction of flavonoids

Collected plant parts were separately shade dried, finely powered using a blender and subjected to extraction following the method of Subramanian and Nagarjan.[30] 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 a separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions

Table 1: Selected plant and parts for flavonoid extraction

Name of plant (Herbarium no.)	Parts	Flavonoid extraction
Anaegissus rotundifolia (RUBL211363)	Root	Free flavonoid
		Bound flavonoid
	Stem	Free flavonoid
		Bound flavonoid
	Bark	Free flavonoid
		Bound flavonoid
	Flower	Free flavonoid
		Bound flavonoid
	Gum	Free flavonoid
		Bound flavonoid

were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2 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

Selected plant parts were taken for flavonoids extraction following the well-established method.[30] Each extract was dried in vacuo and stored at 4°C in airtight vials for further use. Percent (%) extractive values were calculated by the following formula:

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

Drugs and chemicals used

Drugs

Streptomycin, ciprofloxacin, and ceftriaxone (standard antibiotics for bacteria) were used.

Chemicals

Sabouraud dextrose agar medium (peptone 10 g; dextrose 20 g; agar 20 g in 1000 ml of distilled water; pH adjusted to 6.8–7.0 at 27°C ± 2°C), ammonium chloride, ammonium solution, ethyl acetate, and H_2SO_4 were used.

Microorganisms

The organisms used in this study were six Gram-negative bacteria and one Gram-positive bacteria [Table 2]. Selected microorganisms were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on "Mueller-Hinton Agar Medium" (MHA) at 37°C ± 2°C, subcultured regularly (after every 30 days) and stored at 4°C as well as at −80°C by preparing suspensions in 10% glycerol.[32]

Table 2: Selected pathogens (Gram-positive and Gram-negative bacteria)

Name of pathogens	G + ve/G − ve	Specimen no.
Escherichia coli	G − ve	MTCC-46
Raoultella planticola	G − ve	MTCC-530
Bacillus subtilis	G + ve	MTCC-121
Enterobacter aerogenes	G − ve	MTCC-111
Proteus mirabilis	G − ve	MTCC-3310
Klebsiella pneumoniae	G − ve	MTCC-4030
Agrobacterium tumefaciens	G − ve	MTCC-431

Preparation of test pathogens and 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.[33] Bacterial strains were grown and maintained on NA medium. Bacterial growth can observe after a minimum of 18 h and occasionally until 24 h. DDA was performed for screening. NA base plates were seeded with the bacterial inoculum (inoculum size 1 × 10^8 CFU/ml for bacteria). Sterile filters paper discs (Whatman no. 1, 5 mm 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 vacuo so as to remove residual solvent, which might interfere with the determination.[34] Petri plates were pre-seeded with 15 ml of growth agar medium and 1.0 ml of inoculum.[35,36] Extract discs were then placed on the preseeded agar plates. Each extract was tested with streptomycin (10 mcg/disc), ciprofloxacin (10 mcg/disc), and ceftriaxone (10 mcg/disc) commercial discs of antibiotics as positive control (standard), and experiment was done thrice[37] 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).[38] ZOI or depressed growth of microorganisms was measured. This method was followed by various researchers.[39-42]

ZOI

ZOI (mm ± SD), measured in mm (mean value; include 5 mm diameter of disc), indicates that

no bacterial growth around the tested alkaloid extract disc. ZOI measured and compared with the standard reference antibiotics.[43-45]

AI

AI for each extract was calculated by the formula: [46]

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

Determination of MIC by serial dilution method/microbroth dilution (MBD) method

MICs are considered as the "gold standard" for determining the susceptibility of the organisms to antimicrobials.[47] MIC was determined for plant extracts those showing antimicrobial activity against test pathogens in disc diffusion assay.[48-50] Broth microdilution method[51] was followed for determination of MIC values. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 10 mg/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°C ± 2°C for 24 h for bacteria in a biological oxygen demand (BOD) 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[52] 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.[53,54] Broth media of 96-wells of microtiter plates using two fold dilution or serial dilution method. Thereafter, 100 µl 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 the visible growth of microorganisms.

The MIC values were taken as the lowest concentration of the extracts in the test tubes that showed no turbidity after incubation.[55] The turbidity of the test tube was interpreted as the visible growth of microorganisms.

Determination of MBC by serial dilution method/MBD method

It is defined as the concentration of the antimicrobial that results in a 99.9% reduction in CFU/ml compared with the organism concentration in the original inoculum.[56] Equal volume of various concentration of each extract and nutrient agar was mixed in micro tubes to make up 0.5 ml of solution. Then, 0.5 ml of McFarland standard of the organism suspension was added to each tube.[48] The tubes were incubated aerobically at 37°C for 24 h for bacteria in a BOD incubator and observed for change in turbidity after 24 h comparison with the growth and sterility control. 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 subculturing the test dilution on nutrient agar followed by incubation. The MBC was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC.[57] MBC was calculated for those extracts that had shown high antimicrobial activity against tested organisms.

TA (mg/ml)

TA is the volume at which test extract can be diluted without loosing the ability to kill microorganisms.[58] 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/g. In mathematical terms it can be expressed as:

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

Statistical analysis

Mean value and standard deviation were calculated for each test bacteria. Data were analyzed by one-way ANOVA and *P* values ($P > 0.005$) were considered significant.[54]

RESULTS

Preliminary phytoprofiling

The preliminary phytoprofiling (yield and colour) for the different parts (root, stem, bark, flower, and gum) of *A. rotundifolia* was carried out [Table 3] according to Farnworth.[59] The highest yield (w/w in percent) was recorded [Table 3] for alkaloid extraction of gum (3.56%) followed by bark (3.16%).

Antibacterial activity

Alkaloids extracts of all the plant parts were found to possess strong antimicrobial activity against these test pathogens, as revealed by ZOI (mm ± SD) and AI, MIC (mg/ml), MBC (mg/ml), and TA (ml/g) of extracts against each sensitive test pathogens were also evaluated. Alkaloids extracts were screened against selected microorganisms and recorded.

Table 3: Preliminary phytoprofile of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia*

Phytochemical estimation	Root	Stem	Bark	Flower	Gum
% Yield	2.23	2.57	3.16	2.57	3.56
Yield in mg/g dry weight	22.3	25.7	31.6	25.7	35.6
Yield in g (30 g)	0.669	0.771	0.948	0.771	1.068
Colour	Brown	Yellow	Dark brown	Yellow	Light yellow

ZOI (mm ± SD)

Most susceptible organism in the investigation was *B. subtilis* (Gram-positive bacteria) against which, most of the plant extracts (flavonoid extracts) of different parts (root, stem, bark, flower, and gum) of selected plant showed higher ZOI as compare to the other organisms (Gram-negative bacteria). Maximum antibacterial activities were recorded for root and stem extracts of the selected plant. Alkaloid extracts of root and stem showed same value of ZOI (mm ± SD), 16.50 ± 0.24 mm and 16.50 ± 0.21 mm, respectively, against *B. subtilis* (Gram-positive bacteria), followed by gum (16.33 ± 0.24), flower (15.67 ± 0.65), and bark (13.67 ± 0.24) against the above Gram-negative bacteria [Table 4].

AI

Highest AI was recorded for flavonoid extract from root and stem of *A. rotundifolia* against Gram-positive bacteria. Flavonoid extracts of root and stem showed same AI (1.100) against *B. subtilis* (Gram-positive bacteria) followed by gum (1.089), flower (1.045), and bark (0.911) against the above Gram-positive bacteria [Graph 1].

MIC (mg/ml)

MICs were evaluated for those plant parts extracts, which had shown activity in "DDA." The range of MIC of alkaloid extracts recorded was 0.020–10 mg/ml. In the present investigation lowest MIC values (0.078 mg/ml) were recorded for root, stem, and gum followed by flower extract of *A. rotundifolia* against *B. subtilis* [Table 5].

MBC (mg/ml)

The highest dilution that yielded no single pathogen was taken as the MBC. The range

Table 4: ZOI (mm±SD) of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia* against tested pathogens

Pathogens	Root	Stem	Bark	Flower	Gum
Escherichia coli	14.17±0.24	13.33±0.26	11.67±0.65	13.33±0.24	13.67±0.65
Raoultella planticola	11.50±0.21	10.67±0.27	10.33±0.26	12.50±0.24	13.17±0.26
Bacillus subtilis	16.50±0.24	16.50±0.21	13.67±0.24	15.67±0.65	16.33±0.24
Enterobacter aerogenes	12.83±0.66	11.83±0.67	10.50±0.21	11.33±0.24	11.50±0.21
Proteus mirabilis	11.67±0.27	11.17±0.27	10.33±0.27	10.50±0.21	10.83±0.27
Klebsiella pneumoniae	10.83±0.26	9.67±0.26	9.67±0.24	8.83±0.27	11.50±0.21
Agrobacterium tumefaciens	11.50±0.24	10.83±0.24	10.33±0.26	10.50±0.24	10.67±0.26

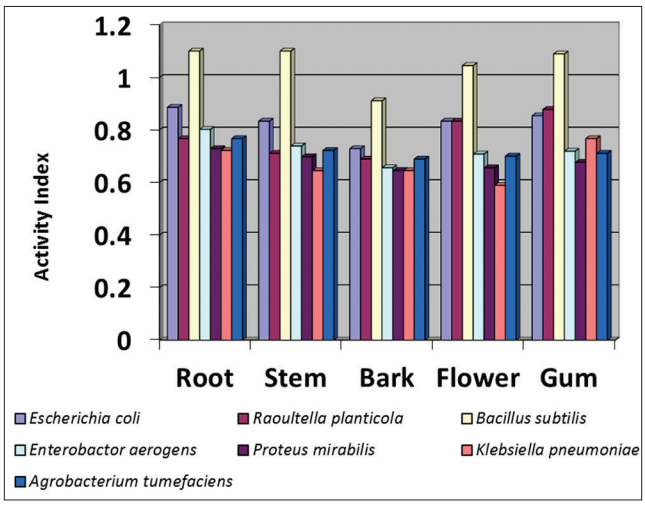
ZOI: Zone of inhibition

of MBC of flavonoid extracts recorded was 0.020–10 mg/ml. In the present investigation lowest MBC values (0.078 mg/ml) were recorded from alkaloid extract of gum of *A. rotundifolia* against *B. subtilis* [Table 6].

MIC and MBC values were found equal for 14 values out of total 35 values (*K. pneumoniae* show 4/5, *R. planticola* show 3/5, *A. tumefaciens* and *P. mirabilis* show 2/5, and *E. coli, Bacillus subtilis,* and *E. aerogenes* show 1/5 times equal) which produce the bactericidal effect [Table 6].

TA (ml/g)

TA indicated the volume up to which the extract could be diluted without losing the ability to kill the microorganisms. In the present investigation higher TA values were recorded for *B. subtilis* (Gram-positive bacteria) as compared to the other organisms (Gram-negative bacteria). Highest value of TA (456.410 ml/g) was recorded for alkaloid extract of gum extracts of the selected plant (*A. rotundifolia*), followed by stem (329.487), root (285.897), fruit (164.744), and bark (100.958) against the above Gram-positive bacteria [Table 7].



Graph 1: Activity index of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia* against tested pathogens (Gram-positive and Gram-negative bacteria)

Table 5: MIC of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia* against tested pathogens

Name of pathogens	Root	Stem	Bark	Flower	Gum
Escherichia coli	0.313	2.500	1.250	2.500	0.313
Raoultella planticola	2.500	1.250	1.250	2.500	0.313
Bacillus subtilis	0.078	0.078	0.313	0.156	0.078
Enterobacter aerogenes	2.500	1.250	1.250	1.250	1.250
Proteus mirabilis	1.250	1.250	2.500	1.250	1.250
Klebsiella pneumoniae	1.250	2.500	2.500	2.500	2.500
Agrobacterium tumefaciens	2.500	1.250	1.250	1.250	1.250

MIC: Minimum inhibitory concentration

DISCUSSION

Phytochemical constituents such as tannins, flavonoids, alkaloids, and several other aromatic compounds of plant that serves as defense mechanisms against predation by many microorganisms, insects, and herbivores. This may, therefore, explain the demonstration of

Table 6: MBC of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia* against tested pathogens

Name of pathogens	Root	Stem	Bark	Flower	Gum
Escherichia coli	0.313	5.000	2.500	5.000	0.625
Raoultella planticola	2.500	2.500	1.250	5.000	0.313
Bacillus subtilis	0.156	0.156	0.625	0.313	0.078
Enterobacter aerogenes	5.000	2.500	2.500	2.500	1.250
Proteus mirabilis	2.500	2.500	2.500	2.500	1.250
Klebsiella pneumoniae	2.500	2.500	2.500	2.500	2.500
Agrobacterium tumefaciens	2.500	2.500	1.250	2.500	2.500

MBC: Minimum bactericidal concentration

Table 7: TA of alkaloids of different parts (root, stem, bark, flower, and gum) of *Anaegissus rotundifolia* against tested pathogens (Gram-positive and gram-negative bacteria)

Name of pathogens	Root	Stem	Bark	Flower	Gum
Escherichia coli	71.246	10.280	25.280	10.280	113.738
Raoultella planticola	8.920	20.560	25.280	10.280	113.738
Bacillus subtilis	285.897	329.487	100.958	164.744	456.410
Enterobacter aerogenes	8.920	20.560	25.280	20.560	28.480
Proteus mirabilis	17.840	20.560	12.640	20.560	28.480
Klebsiella pneumoniae	17.840	10.280	12.640	10.280	14.240
Agrobacterium tumefaciens	8.920	20.560	25.280	20.560	28.480

TA: Total activity

antimicrobial activity by the plant extracts. The demonstration of the antimicrobial activity against both bacteria and fungi 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.

Most susceptible organisms observed in the investigations are *B. subtilis*, *E. coli,* and *E. aerogenes* against which, all the plant extracts showed good antibacterial activities. Highest antibacterial activity shown by root and stem against *B. subtilis*. In the case of Gam-negative bacteria, highest antibacterial activity was shown by root extract *E. coli.*

Gram-positive bacteria *B. subtilis* was the most susceptible organism as compared to the Gram-negative bacteria (*E. coli, R. planticola, E. aerogenes, P. mirabilis, K. pneumoniae,* and *A. tumefaciens*) which supported the finding that plant extracts are usually more active against Gram-positive bacteria than Gram-negative bacteria.[2,17,60-79] Susceptibility differences between Gram-positive and Gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. In the Gram-negative bacterial cell wall, outer membrane appears to act as a barrier to many substances including synthetic and natural antibiotics.[78]

Extracts under this study not only inhibit the bacterial growth but also 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 microorganism is 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 Pharm Industries for preparing plant-based antimicrobials drugs. *A. rotundifolia* easily grows in harsh climatic conditions or xeric conditions and requires less care; hence, its use as raw material for preparing drugs would definitely be economical.

Screening of the plant under investigation (*A. rotundifolia*) so far has not been worked out for alkaloids. Mostly crude extracts have been screened and that too without MIC, MBC/minimal fungicidal concentration (MFC), and TA determination. Such studies could only indicate their antimicrobial potential but are not helpful in establishing them as an alternative for an antibiotic. In the present study, IZ, AI, MIC, MBC/MFC, 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 plant *A. rotundifolia*.

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