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By

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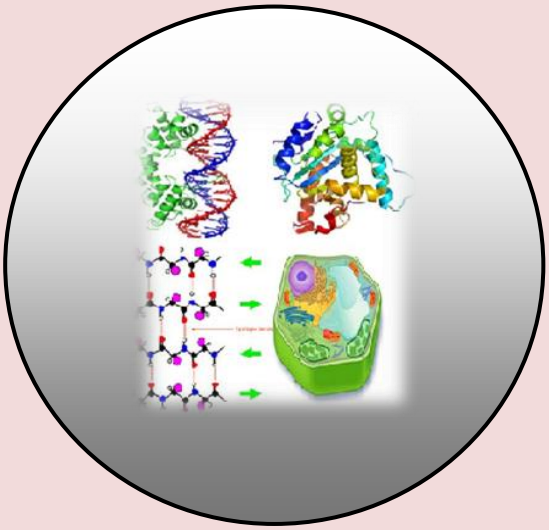
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1), Part C, 2019 Pages No. 116-127



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1, Part C: 116-127, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/4455/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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RESEARCH PAPER

Received: 30/03/2019 Revised: 18/04/2019 Accepted: 19/04/2019

Screening of *Terminalia bellirica, Aegle marmelos, Adhatoda vasica* extract for better control of *Escherichia coli* and *Staphylo coccus aureus* isolated from Urinary Tract Infection

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ABSTRACT

Medicinal herb making its presence in the field of therapy by controlling ever-increasing antibiotic resistance in bacterial species. In the present study, features of three plants named as Terminalia bellirica, Aegle marmelos, Adhatoda vasica systematically investigated for their plant part extracts using petroleum ether, ethanol, methanol and distilled water as a solvent. These extracts were tested to control multidrug resistance Escherichia coli and Staphylococcus aureus isolated from urinary tract infection of a human by well diffusion and synergistic assay tested in vitro. Result showcased that T. bellirica (Behada) fruit extract found to be the most effective extract and able to control MDR strains of E. coli as well as of S. aureus either alone or in synergistic action with antibiotics. It is summarized that developing antibiotic resistance in UTI isolates could be controlled very easily if plant T. bellirica fruit extract used in a systematic way as per our study.
Keywords: Plant extract, multidrug resistance, synergy, E. coli and S. aureus.

INTRODUCTION

World human and animal population is majorly utilizing antibiotics to control the number of bacterial diseases especially at the time of solid organ transplantation, cutting edge surgical practices, cancer treatment and related therapies. But it is important to note that bacterial pathogens are becoming resistant and that leads to ever-increasing mortality (W.H.O., 2014). With the increased antibiotic resistance, the patient also experiences economic burden with different drug use or therapies and as per estimation in the US it is around 20 billion dollar per year (Cosgrove, 2006; Diaz Granados et al., 2005; Sydnor and Perl, 2011). In an alternative approach, traditional medicine finds special place at many times with governed features capable of controlling drug resistance (Ahmad and Beg, 2001; Narayanan et al., 2011; Potroz and Cho, 2015). It has been said that bacteria are incapable of developing resistance towards plant products since it contains many complex phytochemicals and that makes them favourite (Carson and Hammer, 2011). Human suffers disease by many sources among them Urinary tract infection represents its major share and puts up a direct impact on patient health.

Now UTI also carrying a number of Uropathogens tough to control by only antibiotics since they have developed resistance (McLellan and Hunstad, 2016). It is reported that UTI based mortality always remains on the higher side mostly in older adults (Cortes-Penfield et al., 2017). Stamm and Norrby (2001) reported that UTI harbours common bacterial infection with at least 150 million people suffering from the same.

In a view, present study attempted to control ever-increasing MDR *Escherichia coli* and *Staphylococcus aureus* isolated from UTI by using plant extract as next-generation medicine or it may work in synergy with antibiotics by which lost the glory of antibiotics could be regained.

MATERIAL AND METHODS

Isolation of bacterial pathogens from urine

Present study collected urine samples of medically unfit male and female patients visiting local diagnosis laboratory and processed for detection of *Escherichia coli* and *Staphylococcus aureus* presence.

Plating of selective media:

The urine sample was inoculated directly on the Eosin Methylene Blue (EMB) agar for *E. coli* and on Mannitol Salt Agar (MSA) for *Staphylococcus aureus* isolation by spread plate method. After 15 minutes of pause period, plates were shifted in an incubator set at 37℃ for 24 hours. On the next day, obtained colonies were subculture and Gram stained as per standard protocol.

Identification of Bacterial species

Even though EMB and MSA agar allowed selective growth of *E. coli* and *S. aureus,* respectively its identity by molecular level were carried out by targeting 16S rRNA gene sequencing as per the protocol suggested by Rai et al., (2013).

Selection of Plants

In the present study, *A. acanthacae, A. marmelos*and *T. bellirica* plant parts were used to prepare petroleum ether, methanol, ethanol and distilled water based solvent extracts. In a process, 30 ml of solvent was added with 10 grams of plant parts and kept for incubation for 96 hours in a dark at room temperature. It was then filtered by muslin cloth and obtained filtrate was allowed to evaporate in Petri plate when the temperature set at 90℃. Once the solvent evaporated, obtained concentrate was dissolved in 2 ml of 0.9% DMSO and kept in a refrigerator till use.

Antibiotic Sensitivity Assay

Total 16 antibiotics were used for *E. coli* and *S. aureus* antibiotic sensitivity pattern recording by using Kirby Bauer Disc diffusion method. These antibiotics were Polymyxin B (PB 300), Imipenem (IPM 10), Norfloxacin (NX 10), Ceftriaxone (CTR 30), Cefoperazone (CPZ 75), Piperacillin/Tazobactam (PIT 100/10), Mezlocillin (MZ 75), Aztreonam (AT 30), Netillin (NET 30), Carbenicillin (CB 100), Meropenem (MRP 10), Ceftazidime (CAZ 30), Ceftizoxime (CZX 30), Gatifloxacin (GAT 5), Gentamycin (GEN 10) and Tobramycin (TOB 10). Plates were incubated at 37℃ and result was recorded in millimetre as zone of inhibition and as per CLSI chart and those were classified as Sensitive, Intermediate and Resistant.

Antibacterial property of Plant extract

Prepared plant extracts were successfully tested for their individual performance by well diffusion assay. Here *E. coli* and *S. aureus* growing in nutrient broth was set at 1 O.D and used as an inoculum as 100 µl.

In the next step, solidified nutrient agar was firstly inoculated with 100µl of culture broth and spread plate entirely on the plate. It was then allowed to soak on the medium. Thereafter, evenly placed wells were made into the medium. These wells were inoculated with 100µl of DMSO based plant extracts and kept steady for the next 20 minutes. It was then shifted to an incubator set at 37℃ for 24 hours. On the next day, promising extracts able to inhibit bacterial growth of MDR strains was recorded.

Synergism study of antibiotics and plant extract

Once it has been understood that few plants extract capable of inhibiting the growth of MDR *E. coli* and *S. aureus* those were also implemented in synergistic activity along with previously noted antibiotics recorded as resistant. In a study, if plant plus antibiotics action remain capable to improve antibiotic sensitivity it has been considered as positive result.

In a process, firstly molten nutrient agar set at 45°C-50°C was added with 100µl of plant extract and mixed well and allowed to solidify. Nutrient agar was then inoculated with MDR strains of *E. coli* and *S. aureus* and allowed to absorb for 20 minutes. Later on, previously considered resistant antibiotics were placed on the medium aseptically. These plates were allowed to incubate at 37°C for 24 hrs and recorded for the zone of inhibition as per CLSI chart to record the change in sensitivity brought about by synergistic activity.

RESULT

Isolation of *E. coli* and *S. aureus*

As per the routine medium based screening of urine samples of patients; study able to identify 10 *E. coli* and 10 *S. aureus* isolates on the EMB and MSA plates, respectively (Fig. 1). Those were successfully Gram stained as *E. coli* with pink cocco-bacilli and *S. aureus* as a purple rod as shown in Fig. 2.

Antibiotic profiling

As 16 antibiotics tested on 10 isolates of *E. coli* and of *S. aureus* following results were obtained.

Here *E. coli* (n=10) registered 50% resistance to Polymyxin B and Imipenem; 40% to Piperacillin and Norfloxacin; 30% to Ceftriazone, Cefoperazone, Mezlocillin, Carbenicillin, Tobramycin and Gatifloxacin; 20% with Netillin; 10% with Gentamycin and highest resistance up to 70% with Ceftazidime and Ceftizoxime. Here MDR *E. coli* 1 pathogens registered with multidrug resistance towards antibiotics such as Polymyxin B (11 mm); Imipenem (13 mm); Ceftriazone (12 mm); Mezlocillin (17 mm); Aztreonam (NI); Carbenicillin (11 mm); Ceftazidime (NI) and Ceftizoxime (10 mm) as in Table 1 and Fig 3.

In a similar way, *S. aureus* (n=10) reported 50% antibiotic resistance against Polymyxin B; 30% to Meropenem and Mezlocillin; 20% to Imipenem, Gatifloxacin, Tobramycin, Cefoperazone and Pipercillin while antibiotics such as Norfloxacin, Carbenicillin, Ceftizoxime and Gentamycin were found to be sensitive as shown in Table 2.

Here *S. aureus 6* was recorded as MDR strain as it recorded resistance against antibiotics as Polymyxin B (12 mm); Imipenem (11 mm); Aztreonam (NI); and Ceftazidime (9 mm) as in Table 2 and Fig. 4.

Antibacterial activity of plant extract:

In the present study, plant extract prepared from *A. vasica* bark and leaves; *A. marmelos* bark, leaves and fruit and *T. bellirica* bark, leaves and fruit with petroleum ether, ethanol, methanol and water as a solvent. Result showcased that plant extract as 0.03 mg/100 µl able to inhibit *E. coli* and *S. aureus* with a different intensity.

A. vasica (Adulsa) bark extract prepared in petroleum ether (PE), ethanol (e), methanol (m) and aqueous extract (w) did not found to be effective in controlling *E. coli* and *S. aureus* growth (Table 3). In a set of leaf, aqueous extract of it able to inhibit *S. aureus 6* strain with 10 mm of inhibition while *E. coli* remained once again resistant Table 3.

T. bellirica (Behada) bark and leaf extract with all solvents failed to inhibit both *E. coli* and *S. aureus* antibiotic-resistant strains but surely got inhibited profoundly with fruit extract. Here *E. coli* found to be sensitive to the fruit extract prepared in ethanol (13 mm) and water extract (16 mm) while in a set with *S. aureus* Ethanol, methanol and aqueous extract found to be making strain sensitive with 22 mm, 21 mm and 22 mm of inhibition, respectively (Table 3).

In a third set, *A. marmelos* (Bel) found to be totally ineffective to control *E. coli* and *S. aureus* MDR strains with all plant extract tested and strains continue to remain resistant to the plant extract also (Table 3).

Synergistic action of plant extract and antibiotics

Since these three plant extracts of Behada (*T. bellirica*) prepared from fruit extract using ethanol (BeFE), methanol (BeFM) and aqueous source (BeFW) when tested with resistant drugs in many cases profound increase in antibiotic sensitivity was recorded.

Here with Behada fruit ethanol extract, *E. coli* recorded with the higher zone of inhibition when tested in synergy with drug CZX (31 mm), CTR (28 mm), AT (27 mm), CZA (26 mm), CB (23 mm) and least value recorded with PB (12 mm). Overall, it could be put forward that *E. coli* resistance reversal surely been possible when used with fruit extract plus Ceftizoxime (CZX 30). (Table 4) (Fig. 5 and 6).

In a set of Behada fruit extract tested in synergy with antibiotics able to control MDR *S. aureus* profoundly with four antibiotics such as Imipenem, Aztreonam, Ceftazidime, Ceftriaxone and Carbenicillin with more than 40 mm of inhibition indicated the sure success to control *S. aureus* in synergy (Table 4) (Fig. 5 and 6).

In a similar way, Behada fruit extract in Methanol (BeFM) able to control *E. coli* MDR strain proficiently with antibiotic Ceftizoxime (CZX) in synergy and able to showcase zone of inhibition as 33 mm.In the case of *S. aureus* strains, BeFM in synergy with Imipenem able to control the strain with a maximum of 28 mm of inhibition as in Table 4.

Lastly, Behada fruit extract in water able to control *E. coli* with maximum >40 mm of inhibition when tested in synergy with Ceftazidime (CZX) while in the case of *S. aureus,* Behada fruit extract in water did not report to improve the sensitivity of MDR strains and recorded negative (Table 4) (Fig. 5 and 6).

Identification of bacterial species

As per 16S rRNA gene sequence of *S. aureus 6* and *E. coli*, *1* strain those were confirmed to be matching with *S. aureus strain S33* (Accession number NR_037007.2) and *E. colistrain U5141* (Accession number NR_024570.1) as per BLASTN analysis and phylogram, respectively. (Fig. 7 and 8).

DISCUSSION

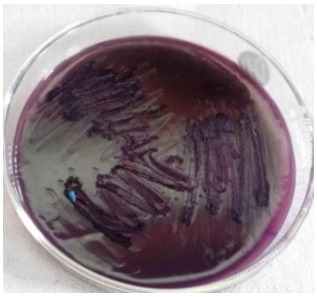
Present study recorded that urine sample of human patients found to be positive for *E. coli* and *S. aureus* species for the number of occasions and those could be easily been sampled by inoculating them on EMB and MSA plates. It is already been evidenced that direct inoculation of the medical sample on eosin methylene blue, Xylose lysine deoxycholate, thiosulfate-citrate-bile salts sucrose, blood agar and mannitol salt agar able to detect a variety of bacterial isolates such as *E. coli, Bacillus sp., Vibrio sp.* and *Staphylococcus sp.* (Sarker et al., 2018). Ghafur et al., (2018) also involved EMB agar to successfully isolate resistant *E. coli* when studied in India. In a similar way, EMB agar was profoundly used in the detection of *E. coli* in the number of medical samples such as urine (Ahmad et al., (2015); Chang et al., (2008).

Similarly, *S. aureus*has been successfully sampled on Mannitol salt agar medium when inoculated with unpasteurized cow milk (Adame-Gómez et al., (2018). Joachim et al., (2018) reported that Health care worker samples; likewise, fermented meat (Stavropoulou et al., 2018); nasal, pigs and land swabs (Founou et al., 2018) to be positive for *S. aureus* when inoculated on Mannitol Salt Agar.

In the present study, *E. coli* strains found to be the most resistant to two antibiotics Ceftazidime and Ceftizoxime by about 70%. Not only is that they registered resistance to the Imipenem, Polymyxin B and Piperacillin. In a similar study, Bryce et al., (2016) carried out 55 massive observational studies and reported 77,738 *E. coli* and out of which 53% recorded resistance to Ampicillin, 23.6% to Trimethoprim. Further, they recorded very high up to 60% for Co-amoxyclove. According to Sidjabat and Pterson (2015), this phenomenon of resistance with *E. coli* has been linked with β-lactamases and found to be producing CTX-M and NDM when investigated in India. Adenipekun et al., (2016) also linked the *E. coli* with multidrug resistance with at least 50% strain resistant to the tetracycline, trimethoprim and ampicillin when investigated in the urine sample. Similar to the present study, Abujnah et al., (2015) reported 6.7 to 15% of ceftriaxone and 23.1% of ciprofloxacin resistance when *E. coli* sampled from urinary tract infection. They also recorded a total of 33.2% of *E. coli* remains multidrug-resistant by possessing the *bloOXA gene*, *blaTEA gene* and *bla-CTX-M gene.*

Similar to the *E. coli*, the present study reported *S. aureus* with 50% resistance in strains for Polymyxin B, 30% resistance to Mezlocillin. Fortunately, the number of antibiotics recorded to be sensitive in the majority of strains such as norfloxacin, carbenicillin, ceftizoxime and gentamycin. It is also noted that 40% of strains attributed as multidrug resistant.

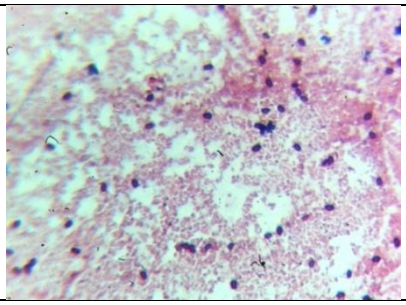
In a similar report, *S. aureus* (2017) grouped as a major public health concern as it harbouring MDR features. They reported massive MDR *S. aureus* prevalence about 82.5% of isolates as MDR with ≥3 antibiotics. Among them, majority showcased ceftazidime resistance (90%), cloxacillin (85.6%), and augmentin (83.3%). Hassanzadeh et al., (2017) linked MDR features of *S. aureus* with several efflux genes such as *nor A, nor B, nor C, mpp A, sep A, mde A* and *smp*and linked those with increased resistance to antibiotic ciprofloxacin.



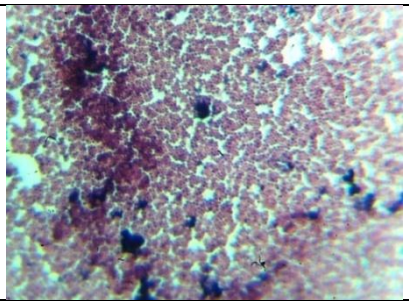
E. coli

S. aureus

Figure 1. Typical colonies of representative isolates recorded on the selective/ differential media.



E. coli



S. aureus

Figure 2. Micrograph of gram staining captured at 100X magnification

Table 1. Antibiotic sensitivity against *E. coli* strains.

Antibiotics	abrevations	Zone of Inhibition (mm)									
		E. coli 1	*E. coli 2*	*E. coli 3*	*E. coli 4*	*E. coli 5*	*E. coli 6*	*E. coli 7*	*E. coli 8*	*E. coli 9*	*E. coli 10*
Polymixin-B	PB300	R	R	S	S	R	S	R	S	R	S
Imipenem	IPM10	R	S	R	S	R	S	R	S	R	S
Norfloxacin	NX10	S	S	R	S	R	S	S	R	S	R
Ceftriaxone	CTR30	R	R	S	S	S	S	S	R	S	R
Cefoperazone	CPZ75	S	S	S	S	S	R	R	S	S	R
Piperacillin/Tazobactam	PIT100/10	S	S	S	S	S	R	R	R	S	R
Mezlocillin	MZ75	R	S	R	R	S	S	S	S	S	S
Aztreonam	AT30	R	S	R	S	S	S	S	S	S	S
Netillin	NET30	S	R	S	S	S	S	R	S	S	S
Carbenicillin	CB100	R	S	S	S	R	S	S	S	S	R
Meropenem	MRP10	S	R	S	S	R	S	S	S	S	R
Ceftazidime	CAZ30	R	R	S	R	R	R	S	R	R	S
Ceftizoxime	CZX30	R	R	R	R	S	R	S	R	R	S
Gatifloxacin	GAT5	S	S	R	S	S	R	S	S	R	S
Gentamicin	GEN10	S	S	S	S	S	S	S	S	R	S
Tobramycin	TOB10	S	S	S	S	R	S	R	S	S	R
Total		16	16	16	16	16	16	16	16	16	16
Sensitive		8	10	10	13	9	12	10	11	10	9
Resistance		8	6	6	3	7	4	6	5	6	7

*Note: S- sensitive; R- resistant; NI- No inhibition

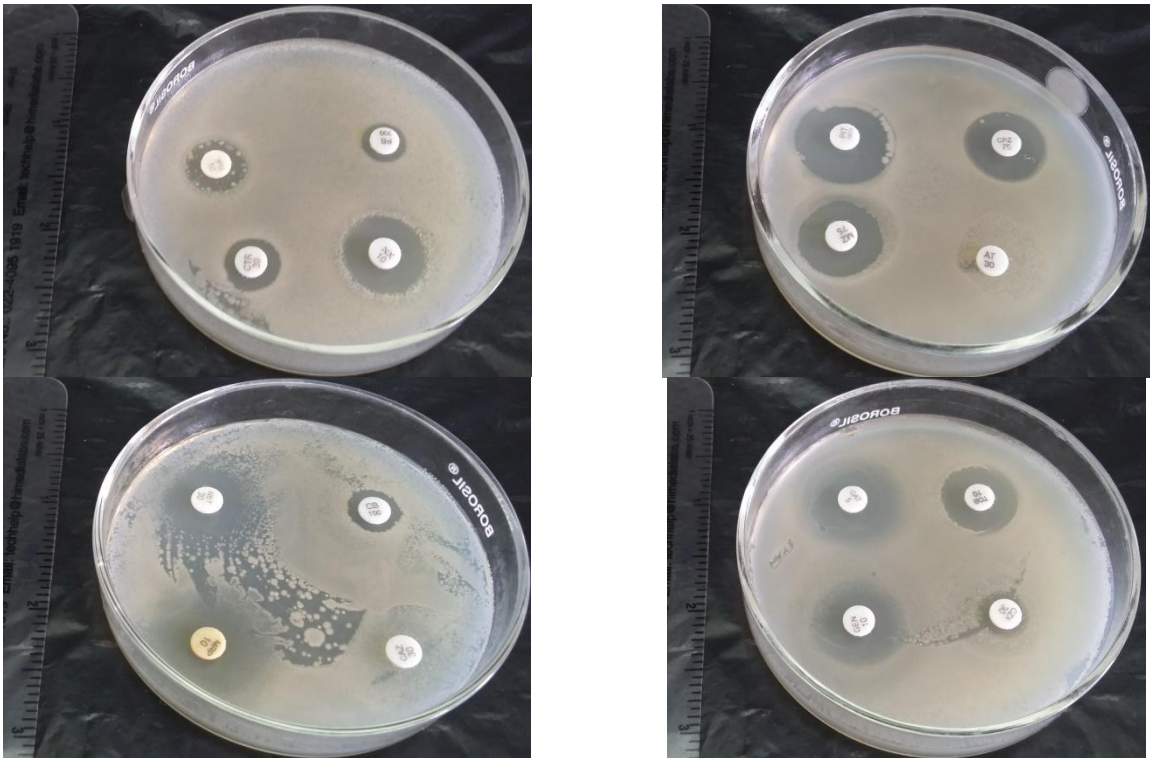


Figure 3. Antibiotic sensitivity against antibiotics of*E. coli strain 1.*

Table 2. Antibiotic sensitivity against *S. aureus* strains.

Antibiotics	abbreviations	Zone of Inhibition (mm)									
		S. aureus									
strains		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Polymixin-B	PB300	R	S	R	S	S	R	S	R	S	R
Imipenem	IPM10	R	S	S	S	S	R	S	S	S	S
Norfloxacin	NX10	S	S	S	S	S	S	S	S	S	S
Ceftriaxone	CTR30	S	S	S	S	S	S	R	S	S	S
Cefoperazone	CPZ75	S	S	S	S	S	S	R	S	S	S
Piperacillin/Tazobactam	PIT100/10	S	S	S	R	R	S	S	S	S	S
Mezlocillin	MZ75	S	S	S	S	S	S	R	S	R	R
Aztreonam	AT30	S	S	S	S	S	R	S	S	S	S
Netillin	NET30	R	S	S	S	S	S	S	S	S	S
Carbenicillin	CB100	S	S	S	S	S	S	S	S	S	S
Meropenem	MRP10	S	S	R	R	R	S	S	S	S	S
Ceftazidime	CAZ30	S	S	S	S	S	R	S	S	S	S
Ceftizoxime	CZX30	S	S	S	S	S	S	S	S	S	S
Gatifloxacin	GAT5	S	R	R	S	S	S	S	S	S	S
Gentamicin	GEN10	S	S	S	S	S	S	S	S	S	S
Tobramycin	TOB10	S	R	S	R	S	S	S	S	S	S
Total		16	16	16	16	16	16	16	16	16	16
Sensitive		13	14	13	13	14	12	13	15	15	14
Resistance		3	2	3	3	2	4	3	1	1	2
*Note: S- sensitive; R- resistant; NI- No inhibition											

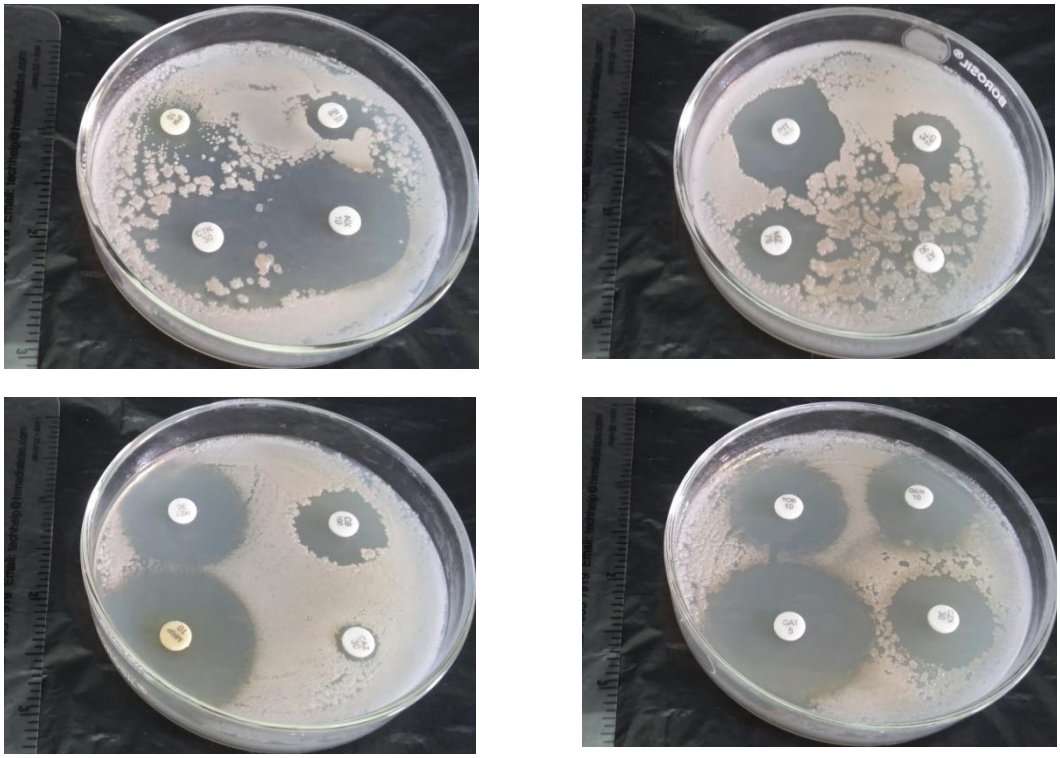


Figure 4. Antibiotic sensitivity against *S. aureus 6.*

Table 3. Antimicrobial activity of Bel, Hirda and Adulsa extract against MDR pathogens.

Sr.No.	Organism	Zone of Inhibition											
		BEL											
		BARK				LEAF				FRUIT			
		BBP	BBE	BBM	BBW	BLP	BLE	BLM	BLW	BFP	BFE	BFM	BFW
1	*E. coli 1*	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
2	*S. aureus 6*	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

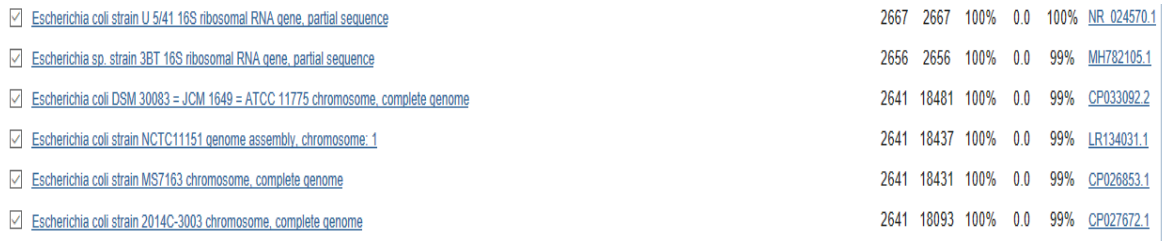
BEHADA											
BARK				LEAF				FRUIT			
BeBP	BeBE	BeBM	BeBW	BeLP	BeLE	BeLM	BeLW	BeFP	BeFE	BeFM	BeFW
NI	NI	NI	NI	NI	NI	NI	NI	NI	13	NI	16
NI	NI	NI	NI	NI	NI	NI	NI	NI	22	21	22

ADULSA							
BARK				LEAF			
ABP	ABE	ABM	ABW	ALP	ALE	ALM	ALW
NI	NI	NI	NI	NI	NI	NI	NI
NI	NI	NI	NI	NI	NI	NI	10

BBP: Bel bark petroleum ether; BBE: Bel bark ethanol; BBM: Bel bark methanol; BBW: Bel bark water; BLP: Bel leaf petroleum ether; BLE: Bel leaf ethanol; BLM: Bel leaf methanol; BLW: Bel leaf water; BFP: Bel fruit Petroleum ether; BFE: Bel fruit ethanol; BFM: Bel fruit methanol;BFW: Bel fruit water; BeBP: Behada bark petroleum ether; BeBE: Behada bark ethanol; BeBM: Behada bark methanol; BeBW: Behada bark water; BeLP: Behada leaf petroleum ether; BeLE: Behada leaf ethanol; BeLM: Behada leaf methanol; BeLW: Behada leaf water;BeFP: Behada fruit petroleum ether; BeFE: Behada fruit ethanol; BeFM: Behada fruit methanol; BeFW: Behada fruit water; ABP: Adulsa bark petroleum ether; ABE: Adulsa bark ethanol; ABM: Adulsa bark methanol; ABW: Adulsa bark water; ALP: Adulsa leaf petroleum ether; ALE: Adulsa leaf ethanol; ALM: Adulsa leaf methanol; ALW: Adulsa leaf water; NI: No Inhibition.

Table 4. Antibiotic sensitivity assay against given plant extracts (synergistic).

Sr. No.	Extracts	Organisms	Antibiotics							
			IPM	AT	CAZ	CZX	PB	CTR	MZ	CB
1	BeFE	*E. coli*	NI	27	26	31	12	28	22	23
2	*BeFE	*S. aureus*	>40	>40	>40		>40			
3	BeFM	*E. coli*	10	29	NI	33	14	24	26	28
4	*BeFM	*S. aureus*	28	NI	14		12			
5	BeFW	*E. coli*	NI	23	25	>40	13	27	26	29
6	BeFW	*S. aureus*	18	12	NI		14			



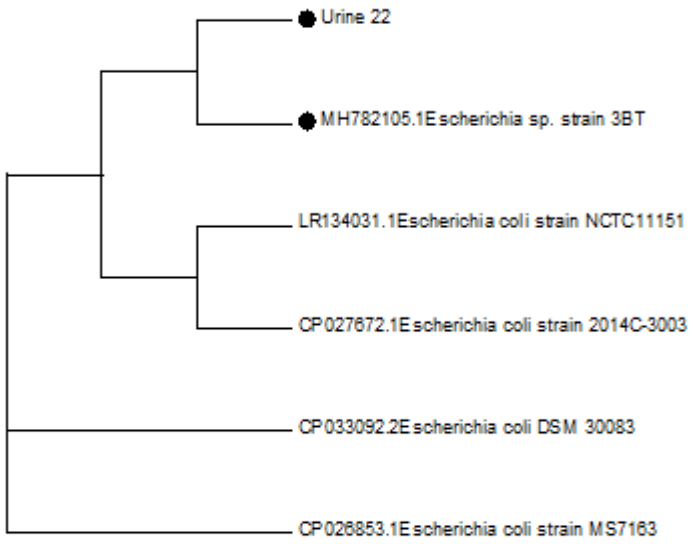
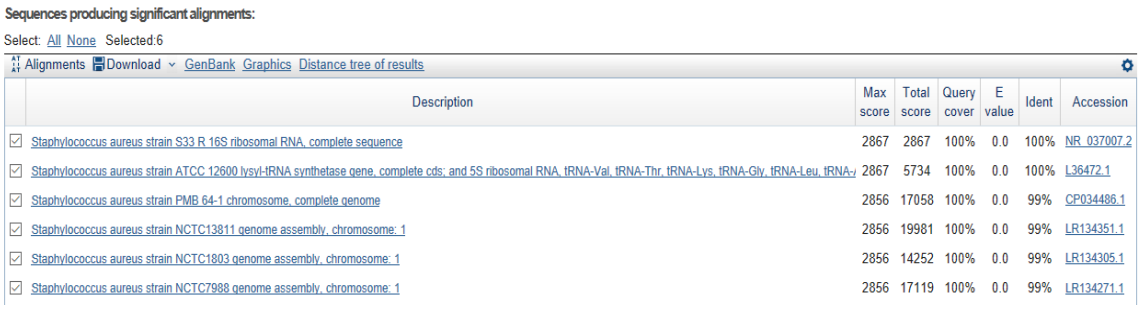


Figure 7. Urine sample idenitfied as *E. coli* confirmed by BLASTN and Phylogenetic analysis.



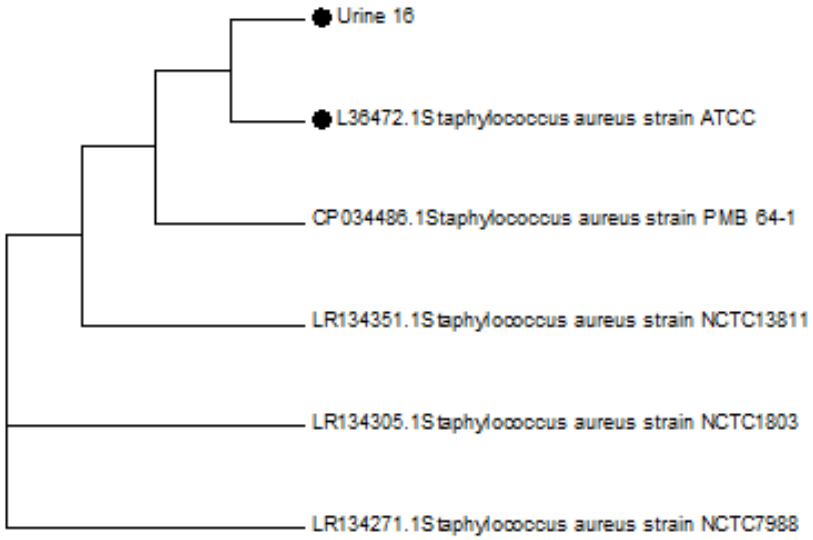


Figure 8. Urinesample idenitfied as *S. aureus* confirmed by BLASTN and Phylogenetic analysis.

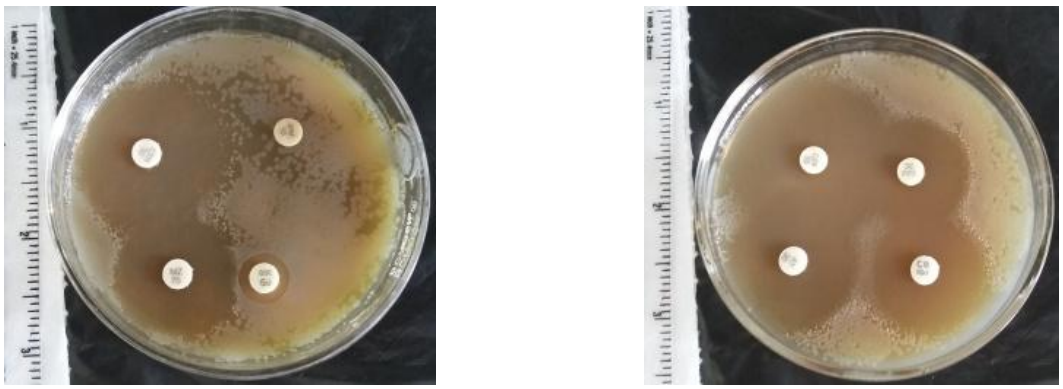


Figure 5a: BeFE.

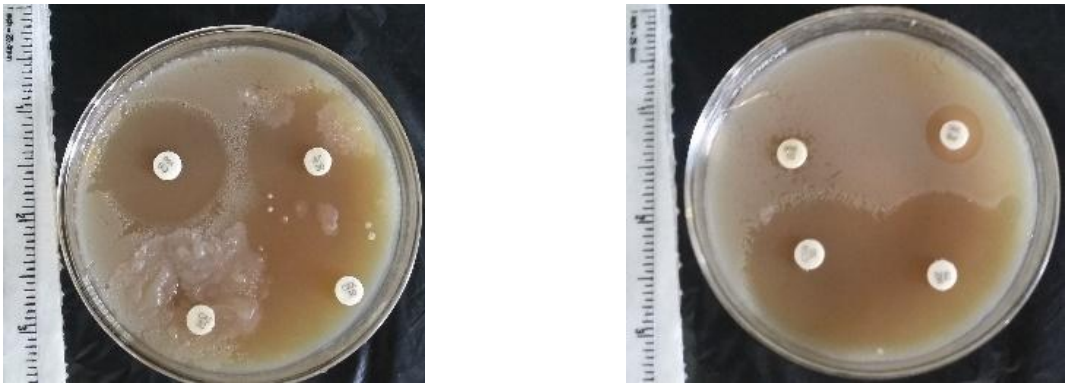
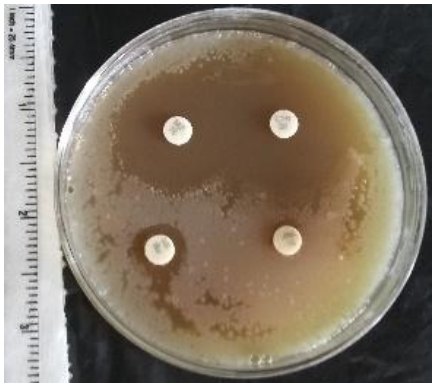


Figure 5b: BeFM.



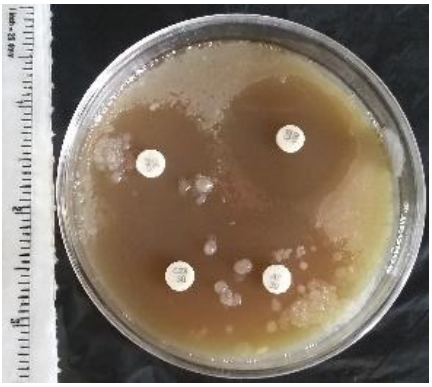


Figure 5c: BeFW.

Figure 5. Synergistic activity of Plant extracts + Antibiotics against *E. coli*

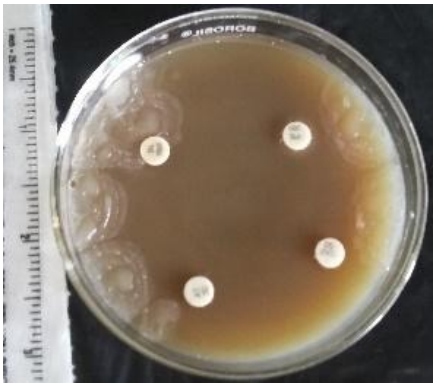


Figure 6a: BeFE

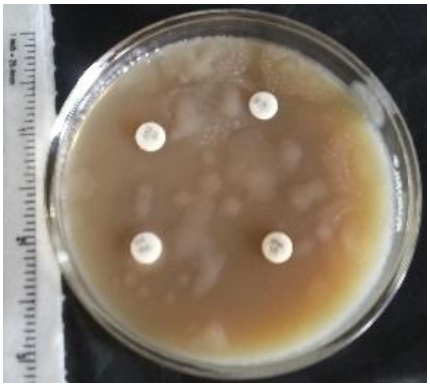


Figure 6b: BeFM.

Figure 6c: BeFW.

Figure 6. Synergistic activity of Plant extracts plus Antibiotics against *S. aureus.*

In the present study, ever-increasing multidrug resistance in *E. coli* and *S. aureus* sampled from urine found to controlled successfully by involving *T. bellirica* (Behada), *A. marmelos* (Bel) and *A. vasica* (Adulsa) plant extracts. It is majorly been observed that *T. bellirica* (Behada) fruit extract overcome the resistance pattern of *S. aureus* and *E. coli* and inhibited majority of multidrug-resistant strains with a maximum of 22 mm of the zone of inhibition. In a related study, Dharmaratne et al., (2018) advocated to use *T. bellirica* in controlling multidrug features of *S. aureus*, along with that of ESBL *E. coli, MDR Acinetobacter sp., Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with given MIC as 0.25/4 mg/ml. In a related report, *T. bellirica* fruit extract based silver nanoparticles also capable of inhibiting *S. aureus* and *E. coli* (Hoskote Anand and Mandal, 2015). Patil et al., (2017) reported synthesising silver based herbometallic colloidal nanosuspension prepared from *T. bellirica* fruit extract by which they controlled *Bacillus subtilis, E. coli, P. aeruginosa* and *S. aureus.*

In the present study, *T. bellirica* (Behada) fruit extract also done potential work in synergistic activity along with the antibiotics with those previously been recorded resistant to the *E. coli* and *S. aureus*. Here *E. coli*, as well as *S. aureus* represented to get inhibited in synergy with plant extract plus antibiotics with maximum >40 mm of inhibition, indicated its sure success in coming time as it was at least double than normal antibiotic activity in general. In a similar study, plant *T. bellirica* at 250 µg/ml worked in synergy reported to be the efficient antimicrobial agent and found to be enhancing the activity of novobiocin at 1 µg/ml (Phatthalung et al., 2012).

CONCLUSION

It is put forward that multidrug-resistantin *E. coli* and *S. aureus* present in Urinary tract could be possible to control if the synergistic or direct application of *T. bellirica*fruit extract (Behada) was implemented. Not only *T. bellirica*but other plants also found to be weakly positive for antibacterial features either alone or in combination with antibiotics and should be advocated for herbal therapy for better control of multidrug resistant activity.

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