In Vitro Antibacterial And Synergistic Effects of *Nyctanthes arbortristis* Leaves Extracts against UTI Pathogens

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Abstract- *The frequent, increasing drug resistance among urinary tract infections (UTIs) causing bacteria has made therapy difficult and also lead to greater use of expensive broad spectrum drugs.The aim of the study was to assess the antibacterial effect of N. arbortristis extracts and their synergistic effect with antibiotics against Escherichia coli and Klebsiella pneumonia. The extract of N. arbortristis were prepared using Soxhlet apparatus. The antibacterial activities of extracts were evaluated using the disk diffusion method. The synergistic effect between plant extracts and antibiotics was also assessed using disk diffusion method.*

The results of this study showed that organic extracts (methanol, ethanol and chloroform) used against uropathogenic isolates were showed antimicrobial and synergistic effect with most antibiotics better than aqueous extract. The methanolic extract of plant exhibited more promising antibacterial activity and synergistic effect with most of the antibiotics, in which gatifloxacin (30.67±0.94mm) and moxifloxacin (30.00±0.00mm) exhibited highest zone of inhibition against E. coli and for K. pneumoniae, amikacin (27.33±0.47mm) showed highest inhibitory effect with the same. This study revealed that K. pneumoniae were slightly less susceptible than E. coli. The combination of plant extracts and antibiotics showed greater zone of inhibition against multidrug resistant bacteria than each of them alone.

The evaluated results exhibited that N. arbortristis extracts have significant potential of antibacterial properties and seems promising for the development of the safe herb derived medicinal preparation for treating UTIs. And these results was exhibited the importance of N. arbortristis extracts when associated with antibiotic in control of Urinary Tract Infections.

Keywords- Plant extracts, Urinary tract infections, Antimicrobial activity, Synergistic effects, Disc diffusion assay, Uropathogen.

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I. INTRODUCTION

Urinary tract infections (UTIs) are broad term used to describe the most commonly encountered bacterial infections affecting human population across the globe. UTIs are a significant cause of morbidity in outpatients as well as most commonly involved in the cause of nosocomial infections in many infirmaries (Abdullahi et al., 2010). A wide range of pathogens responsible for UTIs, but leading etiological agents are *Escherichia coli, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis* (Abiramasundari et al., 2011). *Escherichia coli* is one of the most common urinary pathogen accounting for more than 80% of all community acquired infections (Ghosh et al., 2008). *K. pneumoniae* strains are the second or third most frequent cause of UTIs behind *Escherichia coli*, which causes the vast majority of UTIs (Czaja et al., 2007; Echols et al., 1999; Lorente et al., 2005).Typically, *K. pneumoniae* accounts for 2 to 6% of nosocomial UTIs and 4.3 to7%of community-acquired UTIs (Laupland et al., 2007; Linhares et al., 2013). Appropriate empirical treatment of urinary tract infections (UTIs) is important for the control and prevention of complications.

However, owing to the increasing prevalence of antibiotic-resistant urinary pathogens, the selection of appropriate empirical agents are becoming increasingly difficult. The appearance of new antibiotic resistant bacteria is a societal problem that requires the development of new alternative treatments (Silva et al., 2013). For developing a cheap broad-active agent that can be applicable against different pathogens, it is necessary to develop an alternative source for normal antibacterial agents (Neethu et al., 2013). Herbal medicines represents one of the most important fields of traditional medicine worldwide. A wide range of study on

medicinal plants is essential to determine their potential as a source of new medicine which further promote the use of herbal medicine and their role in upcoming therapies (Kaur and Kaur 2010).

Moreover *Nyctanthes arbor-tristis*is an important medicinal plant belongs to family Oleaceae, commonly known as Parijata (Sanskrit) and Harsingar (Hindi), Night Jasmine (English). It is a small tree with its fragrant flowers found wild in the forests of Central India and Sub-Himalayan regions, cultivated in gardens in many parts of India (Panda 1999). Conventionally the flowers are used as stomachic, expectorant, ophthalmic purpose, skin diseases (Khatune et al., 2003; Sasmal et al., 2007), the stem bark is given in rheumatic joint pain, malaria, bronchitis (Kirtikar et al 1993), leaves are used for treatment of various diseases like sciatica, chronic fever, as a laxative, diaphoretic and diuretic (Tuntiwachwuttiku et al., 2003) and seeds are used as anthelmintics and in alopecia (Nair et al., 2005). Many studies proved that this plant possess various activities such as anti-inflammatory (Singh et al., 2008), hepatoprotective (Vishwanathan et al., 2010), antidiabitic and antioxidant (Husain et al., 2010), antibacterial (Mahida and Mohan 2007), antileishmanial (Tandon et al., 1991) activities. Antimicrobial assessment of aqueous and alcoholic extract of leaves against numerous gram positive and gram negative strains exposed that *Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia. coli, P. marginata* and *Staphylococcus epidermis* were found more susceptible to the aqueous extract. Chloroform and ethyl acetate extracts of fresh and dried leaf, flowers, fruits and seeds are also reported to have a dose dependent antibacterial activity against gram negative bacteria. The activity has been found significant for fresh plant materials than the dried plant parts. The stem bark extracts (petroleum ether, chloroform and ethanol) are found to have potential antimicrobial activity against *S. aureus, Micrococcus luteus, B. subtilis, E. coli, P. aeruginosa, Candida albicans* and *Aspergillus niger* (Aggarwal et al., 2013; Hirapure et al., 2014; Suparna et al., 2014; Balasubramanian 2012; Geethaet al., 2014).

The use of drugs in combinations is one of the strategy employed to overcome resistance. Previous studies showed that the secondary metabolites from plant are good sources for combination therapy, there are a wide range of phytochemicals which act as multidrug resistance modifiers depicted in various reported studies (Hemaiswarya *et al*., 2008; Betoni et al., 2006) demonstrated that plants either contain antimicrobials that can operate in synergy with antibiotics or contain compounds that have no intrinsic antibacterial activity but are able to sensitize the pathogen to a previously ineffective antibiotic.

The current study is based on to evaluate the antimicrobial activity of *Nyctanthes arbortrisis* leaves extracts alone and in combination with appropriate antibiotic to treat infection caused by urinary tract bacteria (*E. coli* and *K. pneumoniae*). This novel concept of synergism can be helpful to minimize drug resistance by enhancing the effect of appropriate drugs.

II. MATERIALS AND METHODS

2.1 Plant material

Fresh leaves of *N. arbortristis* were collected from Swami Vivekanand University Sagar, India. The taxonomic identities of these plants were confirmed by the Botany Department, Swami Vivekanand University Sagar, India. The collected plant materials were washed with clean water and allow to shade dried for about 2-3 weeks and stored at room temperature before the experiments.

2.2 Preparation of extracts

Plant extracts were prepared by Soxhlet extraction method followed by Okeke et al. (2001).

The selected plant materials were ground into coarse powder using an electric blender. Methanol, ethanol, chloroform, and water were selected as solvent of choice for extraction process. 35 gm of powder material (*N. arbortristis* leaves) was uniformly packed in to a thimble and run in Soxhlet extractor. It was exhaustible extracted with 200 ml solvent (methanol, ethanol, chloroform and water) for the period of about 48 hour or 22 cycles or till the solvent in the siphon tube of an extractor become color less. After this step, extract which was collected in a round bottom flask of the soxhlet extractor assembly was filtered with the help of Watmann No. 1 filter paper. Later, the filtered solvent was evaporated from the extract in the vaccum rotator evaporator to get the syrup consistency.

2.3 Collection of samples

The urine samples of UTI patients were collected from Dr Lal Path Lab, Sagar (MP) and Bacterial strains (*Escherichia coli* and *K. pneumoniae)* were isolated and identified by culture characteristics and standard biochemical test as followed by Murray et al. (2007).

2.4 Antibiotic susceptibility test

The antibiotic sensitivity of the isolates was determined by using the Kirby-Bauer disc diffusion method. The bacterial cultures were grown in Brain Heart Infusion broth at 37°C. After 4 hrs of growth, each microorganism, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated by streaking the swab over the entire surface of Mueller Hinton agar plates. The plates were dried at room temperature for 10 min, before placing the antibiotic discs at equidistance. The plates were incubated for 24 h at 37°C and the diameter of zone of inhibition was measured accordingly (Bauer et al., 1966). Tested organisms were classified as sensitive, intermediate or resistant, based on the NCCLS standards (Sockett 2006). Three replicates were maintained for each test solution. A total of 8 antibiotics were used in this study as shown in Table 1.

2.5 Antimicrobial activity of the medicinal plant extracts against multidrug resistant isolates

The plant extracts concentrations used in the study were of 100 mg/ml, 75 mg/ml, 50 mg/ml and 25mg/ml. The antibacterial activities of plant extracts were detected against multi drug resistant isolates namely; *E. coli* and *K. pneumoniae* using disk diffusion method. A suspension of testing microorganisms were spread on Muller Hinton Agar (MHA) medium. The filter paper discs (6 mm in diameter) were placed on the agar plates which was inoculated with the tested microorganisms and then impregnated with 20µl of plant extract. The plates were subsequently incubated at 37ºC for 24 hrs. After incubation the growth inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm (Kumar et al., 2009). A disc of gatifloxacin and amikacin were used as positive control for testing of *E. coli* & *K. pneumoniae* respectively. Moreover four solvents (methanol, ethanol, chloroform, and water) were used as negative controls. Three replicates were maintained for each test solution.

2.6 Synergism between plant extracts and antibiotics against multi drug resistant strains

The combined activity of plant extracts and conventional antibiotics were carried out by disc diffusion method (Jouda et al., 2016). The bacteria at a concentration of 1.5 × 106 cells/ml (adjusted to the 0.5 Mc Farland turbidity standards) were introduced onto the surface of sterile Muller-Hinton Agar (MHA) plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, the antibiotic filter paper disk of 6 mm in diameter were placed on the surface of inoculated Muller Hinton Agar plates and impregnated with 20 µL of known concentration of plant extracts (100 mg/ml, 75mg/ml, 50mg/ml and 25mg/ml). The plates were incubated at 37ºC for 24 h. The diameters of inhibition zones were measured and compared with that of antibiotic alone. Here, both the antibiotic disc and the extract serve as positive controls for comparison of synergistic or additive activity. An increase in zone of inhibition of extract plus standard antibiotic than the alone standard antibiotic were considered as the synergism among the plant extract and antibiotic. Each test was repeated for three times (Elbashiti *et al*., 2011).

III. RESULTS AND DISCUSSION

Bacterial urinary tract infections (UTIs) represents an important cause of morbidity and mortality in long-suffering patients worldwide. Therefore, the development of new antimicrobial agents for the treatment of UTIs infections is of increasing interest.The effectiveness of antimicrobial drugs for the treatment of resistant bacterial infections is limited. New antimicrobials can be developed for suppressing resistant mutants, but this is a difficult and long process. Many studies were carried out to extract and characterize plant products that inhibit the most pathogenic bacteria which are difficult to be effectively treated due to antibiotics limitation and availability (Sayed and Aly 2014 ; Aly and Bafiel 2008). Synergistic combinations could represent therapeutic alternatives for the treatment of resistant pathogenic microorganisms (Musumeci et al., 2003; Duarte et al., 2012). The main objective of the present study was to evaluate the ability of the selected plant extracts to inhibit the growth of pathogenic bacteria with antibiotics and to determine their ability to enhance the activity of antibiotics. According to standards, antimicrobial activity was recorded when the zone of inhibition is greater than 6 mm.

3.1 Evaluation of *N. arbortristis* leaves extracts bioactivity

Most of the tested plant extracts showed antibacterial activity against *E. coli*, and *K. pneumoniae* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth. In this study, all extracts of the plant showed different results.

The results in Tables 2 to 9 indicates that methanolic leaves extract of *N. arbortristis* showed the highest effect against *E. coli and K. pneumoniae*. The crude extract showed 19.00±1.41, 17.67±0.47, 13.00±0.82 and 10.00±0.82 mm zone of inhibition at the concentrations of 100 mg, 75 mg, 50 mg and 25 mg/ml respectively, against *E. coli*. Moreover the zone of inhibition against *K. pneumoniae* at the same concentrations were observed 16.00±1.63, 14.33±0.47, 12.00±0.82 and 7.67±0.47 mm respectively.While low antimicrobial activity was observed by aqueous extract with a zone of inhibition of

12.33±1.25, 10.67±1.25, 8.00±0.82 and 6.67±0.47 mm at the above concentrations against *E. coli*. Whereas at the same concentrations we observed zones of inhibitions of 11.33±1.25, 9.67±1.25, 8.00±0.82 and 6.33±0.47 mm respectively against *K. pneumoniae.* These results showed that methanol was the better solvent for more consistent extraction of antimicrobial substances from medicinal plant compared to other organic solvents and water, which further supported the results obtained in previously reported studies (Abdallah *et al.*, 2009; Zawahry et al., 2013 and Daihan *et al.*, 2013).

In addition the ethanolic extract of *N. arbortristis* showed excellent antibacterial activity against *E. coli* at the above concentrations, it exhibited 18.00±1.41, 17.00±0.82, 12.33±0.47 and 10.00±0.82 mm zones of inhibitions, respectively. Whereas at the same concentrations we measured zones of inhibitions of 15.67±0.47, 14.00±0.82, 9.67±1.25 and 8.00±0.0 mm respectively against *K. pneumoniae*. Our results shows compatibility with the study of Geetha et al. (2014), as they reported that ethanolic extracts showed higher degree of antibacterial activity than aqueous extracts. The ethanolic extract showed the maximum activity against *Staphylococcus aureus* and *Klebsiella pneumonia.* The present study reported that chloroform extract of *N. arbortristis also* exhibited antibacterial activity against *E .coli* at the above concentrations, it shows 17.67±0.94, 15.33±1.25, 11.00±0.82 and 7.67±0.47 mm zones of inhibitions, respectively. Whereas at the same concentrations it has shown zones of inhibitions of 14.67±1.25, 13.33±1.25, 9.67±0.47 and 7.33±0.47, respectively against *K. pneumoniae.* In another study antibacterial activity of various solvent leaf extract of *Nyctanthus arbor tris-tis* were evaluated and reported against *E. coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Corynebacterium glutaminum* and *Pseudomonas aeruginosa.* All three tested extract (acetone, chloroform, and 1% formic acid) were active, but acetone extract was observed as most active extraction. It showed considerable activity against all bacteria as observed by Mahalakshmi et al. (2018).Similar to our results, Paikara et al. (2015) found that chloroform extract of *N. arbortristis* Showed highest antimicrobial activity and petroleum ether showed no antimicrobial activity**.**

3.2 Evaluation of the Synergistic Effect between Plant Extracts and Antibiotics

We tested and evaluated *in vitro* synergism of leaves extracts of *N. arbortristis* and antimicrobial drugs utilized against *E. coli* and *K. pneumoniae* using disk diffusion method. In our study, the plant extracts showed different synergistic ability to inhibit the growth of tested microorganism depending on the method of extraction. Plants antimicrobials have been found to be synergistic enhancers in that they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug. These results were supported by study of Rakholiya and Chanda (2012). Drug synergism between known antibiotics and bioactive plant extracts is a novel concept and it could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) as demonstrated by Adwan and Mhanna (2008).

3.2.1 The methanolic extract and antibiotics:

Table 2 to 3 summarizes the synergistic effect of methanol extracts against *E. coli*. and *K. pneumoniae*. The results showed that methanolic extract of *N. arbortristis* had a synergistic effect with all antibiotics except with ampicillin which had antagonistic effect & was able to suppress the *E. coli* growth and it exhibited the best synergism with Gatifloxacin, with the zone of inhibition of 30.67±0.94, 28.67±0.47, 26.33±0.47, and 19.00±0.00 mm at concentrations of 100 mg, 75 mg, 50 mg, and 25 mg/ml, respectively. Followed by moxifloxacin with zone of inhibition of 30.00±0.00, 27.67±0.47, 24.33±0.47, and 16.33±0.47mm, respectively at the same concentrations. In contrast to *E. coli*, moxifloxacin, gatifloxacin showed antagonistic effect with methanolic extract against *K. pneumoniae*. Our results supported the previous study of Haq (2019) who reported that among tested antibiotics, highest antagonistic effects were shown by moxifloxacin in combination with plant extracts. The methanolic extract give a synergistic effect with cefepime, kanamycin, aztreonam, ceftriaxone/sulbactam and amikacin, while exhibit antagonistic effect with the rest of antibiotics against *K. pneumoniae*. However the strongest synergism was observed with amikacin, with zone of inhibition of 27.33±0.47, 26.33±0.47, 23.33±0.47 and 15.33±0.47, respectively at same concentrations. These results are in agreement with the study of Jouda (2013), where amikacin showed strongest synergistic effect with most of methanol plant extracts. The better synergistic effect was found with *Allium sativum*. In comparison co-trimoxazole, cefotaxime and nalidixic acid, respectivelyshowed weak synergism with methanol extracts**.** Our results shows contradiction with the study of Haroun et al (2016), who reported that the best synergistic effect exhibited with ampicillin against *K. pneumonia* strains and the FIC indice were ranged from 0.25 to 0.5 for resistant strains**.**

3.2.2. The ethanolic extract and antibiotics:

The ethanolic extract had synergistic inhibitory effect against *E. coli* with most antibiotics except with ampicillin, cefepime and amikacin, had antagonistic effects however the

best synergistic effect was found with kanamycin. In which alone kanamycin showed 7.33±0.47 mm zone of inhibition and with combination of ethanolic extract the zone of inhibition were increased by 29.33±0.47, 28.33±0.47, 23.00±0.82, and 15.33±0.47 mm respectively, at the concentrations of 100 mg, 75 mg, 50 mg, and 25 mg/ml (Table 4). In contrast against *K. pneumoniae* it showed highest effect with amikacin with zone of inhibition of 26.67±1.25, 24.33±0.47, 21.33±0.47 and 14.33±0.47 mm respectively, at the same concentrations (Table 5). These results shows compatibility with the findings of Zawahry et al. (2013), they investigated that the *Acacia nilotica* extract had the best synergism with norfloxacin and amikacin against *K. pneumonia*. Apart from this Haroun et al (2016) observed that combination of ethanol extract and amikacin was found to be synergistically effective against MDR Kp1 and ATCC KP. However ciprofloxacin shows additive and indifference effect when combined with all *T. spicata* extracts against resistant and sensitive *K. pneumoniae* strains.

3.2.3. The chloroform extract and antibiotics:

Similar to ethanol extract, against *E. coli*, ampicillin and cefepime showed antagonistic effects with chloroform extract and it showed highest synergistic effect with kanamycin with zone of inhibitin of 27.33±0.47, 24.67±0.47, 21.67±0.47, and 15.67±0.47 mm at the concentrations of 100 mg, 75 mg, 50 mg, and 25 mg/ml (Table 6). Moreover amikacin showed additive affect with the same at 100mg/ml concentration against *E. coli*. The results (Table7) showed that chloroform extract had a synergistic effects with cefepime, kanamycin, gatifloxacin, aztreonam, ceftriaxone/sulbactam and amikacin and had antagonistic effects with ampicillin & moxifloxacin against *K. pneumoniae*. In the present investigation kanamycin showed better synergism with chloroform extract than other antibiotics against *K. pneumoniae*.

3.2.4. The aqueous extract and antibiotics:

The aqueous extract had a synergistic effect with all antibiotics against *E. coli* and with cefepime showed better synergistic activity than other antibiotics with zone of inhibition of 23.00±0.82, 24.33±0.94, 21.33±0.94, and 15.00±0.00 mm at the concentrations of 100 mg, 75 mg, 50 mg, and 25 mg/ml (Table 8). Similar to our results, Danielle et al. (2019) found that the *Plectranthus ornatus* extract exhibited synergism with ampicillin (a β-lactam), kanamycin and gentamicin (aminoglycosides), with 8-fold reductions in the MIC. In our study we observed that kanamycin with water extract showed better synergism than other antibiotics against *K. pneumoniae* (Table 9)**.** Similar to our findings Matu and Van Staden (2003) revealed that aqueous extract was least active in combinatorial treatments and exhibited no synergism against *E. coli*, *E. faecalis*, *P. vulgaris and S. aureus.* Moreover aqueous extracts exhibited lower antibacterial activities compared to methanolic or *n*-hexane extract.

Furthermore, the results in the present investigation showed that the methanol, ethanol, chloroform, and water extracts of *Nyctanthes arbor-tristis* exhibited varying degree of inhibitory effect with or without antibiotics against tested pathogenic strains. *E. coli* was the more susceptible than *K. pneumoniae*. Our findings indicated that at 100 mg/ml concentration, all extracts showed strongest synergism, whereas at 25 mg/ml concentration they exhibited weak synergism or antagonism with antibiotics against MDR isolates.

Table 1: List of Antibiotics

Antibiotics	Antibiotics potency	Manufactured by
Kanamycin (KA)	30 µg	Pathoteq Biological laboratories (PBL), India
Amikacin (AK)	30 µg	Pathoteq Biological laboratories (PBL), India
Ampicillin (AM)	10 µg	Pathoteq Biological laboratories (PBL), India
Ceftriaxone/Sulbactam (CL)	45 µg	Pathoteq Biological laboratories (PBL), India
Gatifloxacin (GF)	10 µg	Pathoteq Biological laboratories (PBL), India
Cefepime(ZX)	30 µg	Pathoteq Biological laboratories (PBL), India
Aztreonam(AC)	30µg	Pathoteq Biological laboratories (PBL), India
Moxifloxacin(ML)	10 µg	Pathoteq Biological laboratories (PBL), India

Table 2: Antibacterial and synergistic effect of *N. arbortristis* methanolic extract against *E. coli*

Solvent used (Methanol)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*E.coli*)									
		100 mg/ml		75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	19.00±1.41	12.33±0.94	17.67±0.47	11.00±0.82	13.00±0.82	10.00±0.00	10.00±0.82	6.67±0.47	0
CEFEPIME (ZX)	7.33±0.47		25.33±0.94		23.33±0.47		20.67±0.47		14.33±1.25	0
KANAMYCIN (KA)	7.33±0.47		21.33±0.47		19.00±0.00		16.00±0.00		11.00±0.00	0
MOXIFLOXACIN (ML)	13.33±0.94		30.00±0.00		27.67±0.47		24.33±0.47		16.33±0.47	0
GATIFLOXACIN (GF)	11.67±0.47		30.67±0.94		28.67±0.47		26.33±0.47		19.00±0.00	0
AZTREONAM (AC)	14.33±0.47		21.00±0.82		19.00±0.82		15.67±0.47		9.67±0.47	0
CEFTRIAXONE/ SULBACTAM (CL)	13.33±0.47		22.33±0.47		19.67±0.47		15.00±0.00		8.00±0.82	0
AMIKACIN (AK)	15.33±0.47		24.33±0.47		21.33±0.47		17.67±0.47		12.00±0.00	0

Value are the mean of three replicates ± S.D, *-Negative control – methanol extract

Table 3: Antibacterial and synergistic effect of *N. arbortristis* methanolic extract against *K. pneumoniae*

Solvent used (Methanol)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*K.pneumoniae*)									
		100 mg/ml		75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	16.00±1.63	14.67±0.47	14.33±0.47	13.00±0.00	12.00±0.82	10.00±0.00	7.67±0.47	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		26.33±1.70		25.33±0.47		22.00±0.00		14.67±0.47	0
KANAMYCIN (KA)	16.33±0.94		21.67±0.47		19.67±0.47		14.33±0.47		10.00±0.00	0
MOXIFLOXACIN (ML)	6.00±0.00		13.67±0.47		13.00±0.00		9.00±0.00		6.00±0.00	0
GATIFLOXACIN (GF)	11.00±0.82		14.00±0.00		13.67±0.47		10.33±0.47		6.67±0.47	0
AZTREONAM (AC)	13.33±0.94		24.00±0.82		20.67±0.47		20.00±0.00		13.00±0.00	0
CEFTRIAXONE/SULBACTAM (CL)	13.33±1.25		21.67±0.47		20.00±0.82		16.00±0.82		11.00±0.82	0
AMIKACIN (AK)	14.33±0.47		27.33±0.47		26.33±0.47		23.33±0.47		15.33±0.47	0

Value are the mean of three replicates ± S.D, *-Negative control – methanol extract

Table 4: Antibacterial and synergistic effect of *N. arbortristis* ethanolic extract against *E. coli*

Solvent used (Ethanol)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*E-coli*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	18.00±1.41	13.33±0.47	17.00±0.82	11.67±0.47	12.33±0.47	8.33±0.47	10.00±0.82	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		12.33±0.47		14.67±0.47		10.67±0.47		7.33±0.47	0
KANAMYCIN (KA)	7.33±0.47		29.33±0.47		28.33±0.47		23.00±0.82		15.33±0.47	0
MOXIFLOXACIN (ML)	13.33±0.94		28.00±0.00		28.00±0.00		24.33±0.47		17.67±0.47	0
GATIFLOXACIN (GF)	11.67±0.47		21.00±0.82		19.67±0.47		16.33±0.47		11.00±0.00	0
AZTREONAM (AC)	14.33±0.47		29.33±0.47		25.67±0.47		21.33±0.47		13.67±0.47	0
CEFTRIAXONE SULBACTAM (CL)	13.33±0.47		21.33±0.47		18.33±0.94		13.00±1.41		9.00±0.00	0
AMIKACIN (AK)	15.33±0.47		15.00±0.82		15.00±0.00		10.00±0.00		6.67±0.47	0

Value are the mean of three replicates ± S.D, *-Negative control – ethanol extract

Solvent used (water)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*K.pneumoniae*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	11.33±1.25	13.00 + 0.82	9.67±1.25	9.33+0.94	8.00±0.82	7.00±0.82	6.33±0.47	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		13.67+0.47		12.33+0.94		10.67+0.94		7.00±0.82	0
KANAMYCIN (KA)	16.33±0.94		18.00+0.82		17.00+0.82		14.67+0.94		12.00±0.00	0
MOXIFLOXACIN (ML)	6.00±0.00		14.67+0.47		12.00±0.00		10.00±0.00		6.33±0.47	0
GATIFLOXACIN (GF)	11.00±0.82		15.33+0.47		13.33+0.47		8.67+0.94		6.00±0.00	0
AZTREONAM (AC)	13.33±0.94		15.00±0.00		15.33+0.47		10.67+0.94		6.67±0.47	0
CEFTRIAXONE SULBACTAM (CL)	13.33±1.25		14.33+0.47		12.00+0.82		10.33+0.94		6.67±0.47	0
AMIKACIN (AK)	14.33±0.47		12.00+0.82		10.00±0.00		9.00±0.00		6.00±0.00	0

Value are the mean of three replicates ± S.D, *-Negative control – water extract

Table 5: Antibacterial and synergistic effect of *N. arbortristis* ethanolic extract against *K. pneumoniae*

Solvent used (Ethanol)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*K.pneumoniae*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	15.67±0.47	14.67±0.47	14.00±0.82	14.33±0.47	9.67±1.25	11.33±0.47	8.00±0.00	7.33±0.47	0
CEFEPIME (ZX)	7.33±0.47		17.00±0.00		15.33±0.47		12.00±0.00		8.67±0.94	0
KANAMYCIN (KA)	16.33±0.94		21.33±0.47		20.00±0.00		17.33±0.47		13.33±0.47	0
MOXIFLOXACIN (ML)	6.00±0.00		24.00±0.47		25.33±0.94		23.33±0.47		14.33±0.47	0
GATIFLOXACIN (GF)	11.00±0.82		24.33±0.47		21.33±0.47		18.33±0.47		11.33±0.47	0
AZTREONAM (AC)	13.33±0.94		23.33±0.47		20.33±0.47		16.33±0.47		7.33±0.47	0
CEFTRIAXONE/ SULBACTAM (CL)	13.33±1.25		21.33±0.94		18.33±0.47		12.33±0.47		6.67±0.00	0
AMIKACIN (AK)	14.33±0.47		26.67±1.25		24.33±0.47		21.33±0.47		14.33±0.47	0

Value are the mean of three replicates ± S.D, *-Negative control – ethanol extract

Table 6: Antibacterial and synergistic effect of *N. arbortristis* chloroform extract against *E. coli*

Solvent used (Chloroform)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*E.coli*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	17.67±0.94	12.67±0.47	15.33±1.25	8.00±0.82	11.00±0.82	7.33±0.47	7.67±0.47	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		14.33±0.47		12.67±0.47		9.67±0.47		6.00±0.00	0
KANAMYCIN (KA)	7.33±0.47		27.33±0.47		24.67±0.47		21.67±0.47		15.67±0.47	0
MOXIFLOXACIN (ML)	13.33±0.94		25.00±0.82		20.33±0.47		17.00±0.82		11.67±0.47	0
GATIFLOXACIN (GF)	11.67±0.47		26.67±0.94		25.67±0.47		20.33±0.47		17.33±0.47	0
AZTREONAM (AC)	14.33±0.47		25.00±0.00		23.00±0.82		18.33±0.47		10.67±0.47	0
CEFTRIAXONE SULB ACTAM (CL)	13.33±0.47		19.33±0.94		19.67±0.47		17.00±0.82		9.67±0.47	0
AMIKACIN (AK)	15.33±0.47		15.33±0.47		13.67±0.47		11.33±0.47		6.33±0.47	0

Value are the mean of three replicates ± S.D, *-Negative control – Chloroform extract

Table 7: Antibacterial and synergistic effect of *N. arbortristis* chloroform extract against *K. pneumoniae*

Solvent used (Chloroform)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*K.pneumoniae*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	14.67±1.25	12.33±0.94	13.33±1.25	11.00±0.82	9.67±0.47	9.00±0.82	7.33±0.47	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		17.00±0.82		15.33±0.47		12.33±0.47		7.33±0.94	0
KANAMYCIN (KA)	16.33±0.94		24.67±0.47		22.67±0.47		19.67±0.47		13.33±0.94	0
MOXIFLOXACIN(ML)	6.00±0.00		14.33±0.47		10.67±0.94		8.33±0.47		6.00±0.00	0
GATIFLOXACIN (GF)	11.00±0.82		17.33±0.94		17.00±0.82		15.33±0.47		7.67±0.94	0
AZTREONAM (AC)	13.33±0.94		18.33±0.94		19.00±0.82		17.00±0.82		11.67±0.47	0
CEFTRIAXONE SULB ACTAM (CL)	13.33±1.25		15.33±0.47		12.00±0.82		9.67±0.47		6.33±0.47	0
AMIKACIN (AK)	14.33±0.47		22.67±0.94		21.00±0.82		18.67±0.47		11.67±0.94	0

Value are the mean of three replicates ± S.D, *-Negative control – chloroform extract

Table 8: Antibacterial and synergistic effect of *N. arbortristis* water extract against *E. coli*

Solvent used (water)	Diameter of growth of inhibition zones (mm) extracts (mg/ml) (*E.coli*)									
	100 mg/ml			75 mg/ml		50 mg/ml		25 mg/ml		
Antibiotics	Antibiotic alone	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	Extract alone	Extract + Antibiotic	*Control
AMPICILLIN (AM)	6.00±0.00	12.33±1.25	12.67+0.94	10.67±1.25	11.00+0.82	8.00±0.82	8.00+0.82	6.67±0.47	6.00±0.00	0
CEFEPIME (ZX)	7.33±0.47		23.00+0.82		24.33+0.94		21.33+0.94		15.00±0.00	0
KANAMYCIN (KA)	7.33±0.47		19.33+0.94		17.33+0.94		14.67+0.47		6.67+0.94	0
MOXIFLOXACIN ML)	13.33±0.94		14.67+0.47		10.67+0.94		7.33+0.47		6.00±0.00	0
GATIFLOXACIN(GF)	11.67±0.47		13.67+0.47		12.67+0.47		9.33+0.94		6.00±0.00	0
AZTREONAM (AC)	14.33±0.47		15.33+0.47		12.67+0.94		11.67+0.47		6.33+0.47	0
CEFTRIAXONE/ SULBACTAM (CL)	13.33±0.47		16.00±0.00		14.67+0.47		10.67+0.94		7.33+0.94	0
AMIKACIN (AK)	15.33±0.47		21.67+0.47		18.00+0.82		15.67+0.47		12.00±0.00	0

Value are the mean of three replicates ± S.D, *-Negative control – water extract

Table 9: Antibacterial and synergistic effect of *N. arbortristis* water extract against *K. pneumoniae*

IV. CONCLUSION

Organic plant extracts were showed antimicrobial and synergistic activity with antibiotics better than aquatic extracts. The strongest effect agaist *E. coli* was recorded when organic plant extracts was mixed with gatifloxacin, moxifloxacin, kanamycin and amikacin. Moreover the strongest effect on *K. pneumoniae* was observed when organic plant extracts was combined with amikacin, kanamycin and aztreonam. The results of this research work have revealed the synergistic effect from the association of antibiotics with plant extracts against resistant bacteria this may leads to new choices for the treatment of infectious diseases, which enables the use of a mixture of antibiotics and plant extracts against bacterial infections during therapeutic treatment when it is no longer effective by itself against bacterial infections.

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