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**ARTICLE**

- Antimicrobial activity of secondary metabolites obtained with different carbon sources at different stages of the *Pseudomonas* growth curve isolated from Fresnillo, Zacatecas, México mineral soils** 30  
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## Full Length Research Paper

# Studies on antibacterial spectrum of extracts of stem bark of *Azadirachta indica* A. Juss on some clinical isolates associated with urinary tract infection

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This investigation was aimed at determining the antibacterial activity of extracts of stem bark of *Azadirachta indica* A. Juss on some clinical isolates. Results of preliminary analysis showed that the stem bark of *A. indica* possesses alkaloids, flavonoids, tannins, cardiac glycosides, phlobotannins and phenols. The ethanol extract produced higher antibacterial activity against all test microorganisms compare to the acetone extract, the highest *Proteus vulgaris* (15 mm) with ethanol extract at 500 mg/ml, and *Staphylococcus aureus* (13.5 mm) with acetone extract at 500 mg/ml respectively. Both the extracts produced definite strong antibacterial activity against test organisms at 50 to 1000 mg/ml concentration. Statistical analysis ( $t_{cal} = -0.42$ ) revealed that there was no significant difference among the antibacterial activities of ethanol and acetone extracts of *A. indica* against the test organisms. At pH value 2.0, the antibacterial activity of ethanol extract at 500 mg/ml was increased significantly against *P. mirabilis* (diameter of zone 20.0 mm) compared to the untreated one at pH 4.2 (diameter of zone 9.0 mm). In the case of *S. aureus* at pH 8.0 there was an increase in antibacterial activity of the ethanol extract (diameter of zone 29.0 mm) when compared to untreated extract at pH 4.2 (diameter of zone 14.0 mm). The minimum inhibitory concentration (MIC) values for acetone extract ranged from 100 to 500 mg/ml for the test organisms, and for ethanol extract the value was 200 mg/ml for all the test organisms. In case of test organisms, the minimum bactericidal concentration (MBC) value was 500 mg/ml for both extracts. So, the use of *A. indica* for treatment of urinary tract infection may be justified.

**Key words:** Phytochemicals, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), *Azadirachta indica*, *Proteus mirabilis*.

## INTRODUCTION

Treatment using medicinal plants started since the beginning of history. People of ancient times strongly

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relied on the healing power of plants, and extensively used them for various ailments (Khattak, 2012). Medicinal plants are the most productive source of new drugs of natural origin (Ayo et al., 2013). There are numerous medicinal plants used in the World like *Aloe vera* leaves that contain phytochemicals, which are studied for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, other anthraquinones, such as emodin, and various lectins (Boudreau and Beland, 2006).

Hexane, ethyl acetate, ethanol and methanol extracts of *Psidium guajava* were tested against the dental caries causing bacteria *Streptococcus mutans* and fungus *Candida albicans* isolated from caries infected patients, all the four extracts of *P. guajava* showed activity against both *S. mutans* and *C. albicans*. *Terminalia chebula* and *Mimusops elengi* showed antibacterial activity against *S. Mutans* (Jebashree et al., 2011).

In South Africa, the ethanol extracts of the following plants *Annona senegalensis*, *Englerophytum magalismontanum*, *Dicerocarym senecioides*, *Euclea divinorum*, *Euclea natalensis*, *Solanum panduriforme* and *Parinari curatellifolia* showed antimicrobial activity against the following organisms, *Actinobacillus actinomycetemcomitans*, *Actinomyces naeslundii*, *Actinomyces israelii*, *Candida albicans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *S. mutans* (More et al., 2008).

*Zingiber officinale* and *Allium sativum* extracts (Water and Ethanol) were investigated on selected food-borne pathogens that is, *Escherichia coli*, *Salmonella species*, *Staphylococcus aureus* and *Bacillus cereus*, and results obtained showed resistance from all microorganisms except *Escherichia coli* aqueous extract and ethanol extract only against *S. aureus* and *S. species* (Ogodo and Ekeleme, 2013). *Achillea millefolium* found mostly in Brazil has essential oils gotten from its stem and leaves present a very high antimicrobial activity against *Streptococcus pneumoniae*, *Clostridium perfringens* and *Candida albicans*, and slightly inhibited *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krusei* (Silva and Fernandes, 2010).

In Kenya, medicinal plants like *Withania somnifera*, *Warbugia ugandensis*, *Prunus africana* and *Plectranthus barbatus* were used to check antimicrobial and antifungal activities against *B. cereus*, *S. aureus*, *Mycobacterium luteus*, *Pseudomonas aeruginosa*, *C. albicans*, *Cryptococcus neoformans*, *Microsporum gypseum*, and *Trichophyton mentagrophytes*, and it proved positive (Mwitari et al., 2013).

Panghal et al. (2011) carried out an experiment using the oral sample from 40 cancer patients to deduce the microorganisms and their reaction to 10 medicinal plants. The microorganisms that were identified were *S. aureus* (23.2%), *E. coli* (15.62%), *Staphylococcus epidermidis* (12.5%), *P. aeruginosa* (9.37%), *Klebsiella pneumonia*

(7.81%), *Proteus mirabilis* (3.6%), *Proteus vulgaris* (4.2%) and the fungal pathogens were *C. albicans* (14.6%), and *Aspergillus fumigatus* (9.37%). Out of 40 cases, 35 (87.5%) were observed as neutropenic and 8 medicinal plants showed great antimicrobial activity (*Asphodelus tenuifolius*, *Asparagus racemosus*, *Balanites aegyptiaca*, *Eucalyptus alba*, *Murraya koenigii*, *Pedaliium murex*, *Ricinus communis* and *Trigonella foenum graecum*).

Antibacterial effects of aqueous and ethanolic extracts of seeds of *Moringa oleifera* and pods of *Annona muricata* (which contains Quinolines and isoquinolines, Annopentocins, Annomuricins, Coreximine and reticuline) were examined against *S. aureus*, *E. coli* (isolated from White-leg shrimp and the aquatic environment), *Vibrio cholerae* and *Salmonella enteritidis*. Both extracts of *M. oleifera* and ethanolic extracts from *A. muricata* showed antimicrobial activities against stated microorganisms (Mourao et al., 2010).

Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycoacterium tuberculosis* and streptomycin resistant strains. *In vitro*, it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae*, *M. tuberculosis* and *Mycoacterium pyogenes*, *Neisseria gonorrhoeae*, the multidrug resistant *S. aureus* and urinary tract *E. coli*, *Salmonella typhi*, *Salmonella paratyphi*. Antimicrobial effects of neem extract have been demonstrated against *S. mutans* and *S. faecalis* (Adyanthaya et al., 2014).

Leaves of *Azadirachta indica* possess antibacterial, antifungal, antiviral and even antiparasitic property, and therefore exhibit wide range of pharmacological activities of neem leaf which may include immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Subapriya et al., 2005). The leaf extracts of the plant exhibit antidermatophytic activity against dermatophytes such as *Trichophyton rubrum*, *Mentagrophytes*, *Tricophyton violaceum*, *Microsporum nanum* and *Epidermophyton floccosum* (Heinrich et al., 2005).

*Saraca asoca* exhibit antibacterial and antifungal activities against several bacteria: *Bacillus subtilis*, *E. coli*, *Salmonella typhosa* and *S. aureus* and fungi *Alternaria cajani*, *Helminthosporium sp.*, *Curvularia lunata* and *Fusarium sp.* (Pradhan et al., 2009). Neem leaves have antibacterial properties, and could be used for controlling airborne bacterial contamination in the residential premises (Khan et al., 2008; Mahmood et al., 2010) supports the use of the Neem seeds in traditional medicine to treat infectious conditions especially those involving the eye and ear (Amer et al., 2010). The aim of this research work was to determine the antibacterial activity of extracts of stem bark of *A. Indica* against several clinical isolates.

## MATERIALS AND METHODS

### Collection of stem bark of *A. indica*

The stem bark of *A. indica* was obtained from the premises near Lecture Theater 2, Covenant University. The plant was identified, and authenticated by Dr. Conrad Omohinmin, Department of Biological Sciences Covenant University. The sample was collected on 10th December, 2014.

### Preparation of samples

The stem bark of *A. indica* was dried under the laboratory conditions. The dried sample was then crushed using mortar and pestle; later, the crushed materials were further ground to powdered form by electric blender (National model NO. Mx-795N-1 Litre). This was done to enhance the penetration of the extracting solvents into the cells in order to facilitate the release of active principles (Jigna and Chanda, 2006). The ground samples were then used for extraction purpose.

### Extraction procedure

Ethanol and Acetone were employed as solvents for extraction purpose. Ethanol is used for its broad spectrum, and relative non selective property of extraction and acetone were used because of its selective property of extracting tannins (Jigna and Chanda, 2006). Hot extraction method using the Soxhlet apparatus was used for both ethanol and acetone using 200 g each per sample. Acetone was set at 52°C and Ethanol at 60°C. After using the hot extraction method, the rotary evaporator was employed next to remove the plant extract from the solvent.

### Qualitative phytochemical analysis

The extracts were subjected to qualitative phytochemical tests to determine tannin, phenols, saponin, alkaloids, cardiac glycosides, flavonoids, anthraquinones and phlobatannin using standard procedures as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993).

### Test organisms

The test organisms used in this study were local strains of *Proteus mirabilis*, *P. vulgaris*, *Staphylococcus aureus* and *Bacillus sp.* which were obtained from stock cultures isolated in Jawako Specialist Hospital, Jos Road, Kaduna. The microbial cultures were sub-cultured into appropriate agar slants (*P. vulgaris* and *P. mirabilis* on Salmonella-Shigella Agar, *S. aureus* on Blood agar and *Bacillus spp.* on Nutrient Agar). The isolates were identified by standard microbiological procedures as described in Cheesbrough (2010). Isolates were further identified with the API identification system (Bio-Merieux, Saint Vulbas, France) and KB004 HiStaph™ Identification Kit (Benson, 2005). The isolates were then kept at 4°C for further research work.

### Determination of antimicrobial activity (zone of inhibition) of extracts using agar diffusion method

A loopful of growth of each isolate on agar medium was suspended in sterile water, and then was diluted in steps of 1:10 to give

turbidity equivalent to the 0.5 McFarland standard (a density of  $1 \times 10^8$  cells/ml) before inoculation (NCCLS, 2002). Mueller-Hinton agar was inoculated with 0.5 ml suspension of each isolate adjusted to  $1 \times 10^8$  cells/ml using swab sticks. Gram positive and Gram Negative antibiotic disks were employed as control. About 10 g of the dried acetone and ethanol extracts of stem bark of *A. Indica* were reconstituted with 10 ml of Dimethyl Sulfoxide to get a concentration of 1000 mg/ml used as stock solution. 500, 200, 100, 50 and 25 mg/ml were prepared from the stock solution, and used for the susceptibility test on the isolates. Mueller Hinton Agar (MHA) was the medium used for the test. The medium was prepared according to manufacturer's instructions, and was poured (about 25 ml of the media) into each of the sterile petri-plates. The plates were then allowed to solidify, and a sterile swab stick was used to pick the inoculum suspension and was spread uniformly on the agar. 9 mm cork borer was used to dig a hole on the agar, and the reconstituted extracts were loaded in the hole and antibiotic disks placed on a different plate with microorganisms spread. The extracts and the antibiotics were allowed to diffuse into the agar for 1 to 2 h on laboratory bench. The plates were then incubated for 18 h at 37°C. At the end of incubation, zones of inhibition formed around the hole were measured in mm, and were reported.

### Antibiotic sensitivity test

The isolates used for this test were inoculated using streak plate method in nutrient agar medium, and allowed to incubate for 18 h. Sensitivity disks containing conventional antibiotics such as Augmentin (20 µg), Amoxicillin (10 µg), ciprofloxacin (5 µg), Cotrimoxazole (30 µg), gentamicin (10 µg) and Nitrofurantoin (300 µg) manufactured by Abtek Biological Ltd., England were used to carry out the sensitivity test. A colony of the 18 h culture was picked with an inoculating loop from each isolate on nutrient agar, and was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of  $1 \times 10^8$  cells/ml) before inoculation (Benson, 2005). Mueller-Hinton agar was inoculated with 0.5 ml suspension of each isolate adjusted to  $1 \times 10^8$  cells/ml using sterile spreader. Sensitivity discs containing antibiotics were placed on the surface using sterile forceps of each Mueller-Hinton agar plate evenly seeded with test organisms, and it was incubated for 24 h at 37°C.

### Effect of pH on the antimicrobial activity of acetone and ethanol extract of *A. indica* on microorganisms

The experiment was performed using the method of Emeruwa (1982). The concentrations of extract used for this purpose were 1000 and 500 mg/ml. Each of the extracts of different concentrations was adjusted to pH 2.0 and pH 8.0 with pH meter using 1(N) HCl and 1(N) NaOH respectively, and their antimicrobial activity were determined using agar diffusion method as described earlier.

### Determination of minimum inhibitory concentration (MIC) of extracts (stem bark)

Acetone extract and ethanol extract of the stem bark were used for this purpose. The MIC of the extract was determined using all the named microorganisms. About 1 ml of the varying concentrations (500, 200, 100, 50 and 25 mg/ml) of the reconstituted extract was measured into test tubes containing 1 ml of Nutrient broth (NB) (Aliyu et al., 2009). 1 ml of the test organism previously adjusted to the concentration of  $10^8$  cfu/ml was added to the broth media in



**Table 1.** Preliminary phytochemical screening of sample.

Component	Observation (stem bark)
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Cardiac Glycosides	+
Phlobotannins	+
Anthraquinone	-
Phenols	+

+: Present; -: Absent.

each of the test tubes (Jigna and Chanda, 2006). All the tubes were then incubated at proper temperature for 18 to 24 h. A set of test tubes containing broth only used control were seeded with the same test microorganism as described earlier, and were incubated. The highest dilution of the extract that showed no visible growth of the test microorganism was taken as the MIC.

#### Determination of minimum bactericidal concentration (MBC) of extracts

For each of the test tubes in the MIC that showed no visible growth, a loopful of broth was collected from those tubes and streaked on sterile nutrient agar plates. The plates were incubated at 37°C for 24 h (Jigna and Chanda, 2006).

## RESULTS

#### Characteristics of extracts obtained from stem bark of *A. indica*

For acetone extraction, a reddish brown solid substance was obtained weighing 35.44 g per 200 g of dried stem bark. For ethanol extraction, a reddish brown solid substance was also obtained weighing about 36.53 g per 200 g of dried stem bark. The pH of the extracts was in the range of 4.2 to 4.5.

#### Qualitative phytochemical screening

- (a) Alkaloids: Appearance of buff-colored precipitate indicated the presence of alkaloids.
- (b) Flavonoids: Green-blue coloration was seen indicating the presence of flavonoids.
- (c) Tannins: An occurrence of a blue- black precipitate indicates the presence of tannins.
- (d) Cardiac Glycosides: Development of reddish brown colour at the interphase of chloroform, and sulphuric acid indicated the presence of cardiac glycosides.
- (e) Phlobotannins: Development of red precipitate indicated the presence of phlobatanins.

(f) Phenols: Deep bluish- green colour indicated the presence of phenols.

(g) Saponins: Absence of frothing showed absence of saponins in sample.

(h) Anthraquinone: Absence of Pink or Violet coloration showed absence of anthraquinone

The results are shown in Table 1.

#### Antimicrobial activity of extracts

All the extracts showed measurable diameter of zone at 50 to 1000 mg/ml (for acetone extract 6 to 14 mm for acetone extract and 4 to 15 mm for ethanol extract). The acetone extract at 500 mg/ml showed highest antibacterial activity against *S. aureus* (13.5 mm) followed by *P. vulgaris*, *Bacillus* sp. (13mm), and *P. mirabilis* (11 mm). The ethanol extract at 500 mg/ml showed highest antibacterial activity against *P. vulgaris* (15 mm) followed by *P. mirabilis*, *Bacillus* sp. (14 mm), and *S. aureus* (12 mm). The results are expressed in Table 2. Statistical analysis (t test) for comparing the antibacterial activity of ethanol extract with that of acetone extract shows that there was no significant difference among the two extracts against the test organisms ( $t_{cal.} -0.42$ ).

#### Antibiotic sensitivity test

*P. mirabilis* showed significant antibacterial activity against gentamycin (30 mm) followed by ofloxacin, nalidixic acid (28), Augmentin (27) and nitrofurantoin (24). It did not show any antibacterial activity against cotrimoxazole, tetracycline and amoxicillin. *P. vulgaris* showed significant antibacterial activity against tetracycline, ofloxacin (25) followed by nitrofurantoin, gentamycin (24), augmentin, amoxicillin (23) and nalidixic acid (16). *P. vulgaris* did not produce any measurable zone of inhibition against cotrimoxazole. *Bacillus* sp.

**Table 2.** Antimicrobial activity of extracts (zones of inhibition in mm) against different organisms.

Variable	25 mg/ml		50 mg/ml		100 mg/ml		200 mg/ml		500 mg/ml		1000 mg/ml	
	A	E	A	E	A	E	A	E	A	E	A	E
<i>P. mirabilis</i>	N	N	6.0	7.0	8.0	8.0	11.0	9.0	11.0	14.0	10.0	9.0
<i>P. vulgaris</i>	N	8.0	9.0	11.0	10.0	12.0	13.0	11.0	10.0	15.0	14.0	13.0
<i>S. aureus</i>	N	5.0	5.0	7.0	10.0	9.0	12.0	10.0	13.5	12.0	12.0	11.0
<i>Bacillus spp.</i>	N	N	4.0	5.0	7.0	8.0	10.0	9.0	13.0	14.0	13.0	12.0

N = No measurable zone of inhibition; A = Acetone extract; E = Ethanol extract.

**Table 3.** Diameter of zone of inhibition (mm) of *P. mirabilis*, *P. vulgaris*, *Bacillus sp.* and *S. aureus* against some antibiotics.

Organism	NIT	GEN	COT	TET	AMX	OFL	AUG	NAL	CXC	CHL	ERY
<i>P. mirabilis</i>	24	30	N	N	N	28	27	28	-	-	-
<i>P. vulgaris</i>	24	24	N	25	23	25	23	16	-	-	-
<i>Bacillus sp.</i>	-	28	N	32	N	-	N	-	N	N	24
<i>S. aureus</i>	-	19	N	17	N	-	27	-	20	N	20

N-no measurable zone "-"not carried out; NIT- nitrofurantoin; GEN- gentamycin; COT- cotrimoxazole; TET- tetracycline; AMX- amoxicillin; OFL-ofloxacin; AUG- Augmentin; NAL- nalidixic acid; CXC-chloxacillin; CHL-chloramphenicol; ERY-erythromycin.

**Table 4.** Effect of pH on the potency of the extract.

Variable	500 mg/ml						1000 mg/ml					
	Acetone			Ethanol			Acetone			Ethanol		
	2.0	4.50	8.0	2.0	4.20	8.0	2.0	4.50	8.0	2.0	4.20	8.0
<i>P. mirabilis</i>	16	11	7	20	9	11	15	10	27	11	9	10
<i>P. vulgaris</i>	9	14	11	11	15	9	18	14	10	24	13	13
<i>S. aureus</i>	10	13.5	22	12	14	29	19	12	18	19	11	23
<i>Bacillus spp.</i>	10	11	15	12	10	14	14	13	11	16	12	17

showed definite strong antibacterial activity against tetracycline (32) followed by gentamycin (28) and erythromycin (24). It did not show any antibacterial activity against augmentin, chloxacillin, chloramphenicol, cotrimoxazole and amoxicillin. *S. aureus* showed significant activity against augmentin (27) followed by chloxacillin, erythromycin (20), gentamycin (19) and erythromycin (17). It did not show any activity against chloramphenicol, cotrimoxazole and amoxycillin. The results are shown in Table 3.

#### Effect of pH on the antimicrobial potency of the extract

The antibacterial activity of the extracts increased significantly against *S. aureus* and *P. mirabilis* although there were cogent inhibitions in other microorganisms for

both 2.0 and 8.0 reconstituted extracts as against the untreated samples. The results are shown in Table 4.

#### Determination of MIC and MBC of extracts (stem bark)

The results are shown in Table 5.

#### DISCUSSION

Results of the phytochemical screening indicated the stem bark of *Azadirachta indica* contains some active chemical compounds like cardiac glycosides, phlobotannins, phenols etc. which may be the resulting effect of the antibacterial activity.

Akiyama et al. (2001) reported that Tannic acid is a successful adjuvant agent against *S. aureus* skin

**Table 5.** Determination of MIC and MBC of plants extracts.

Variable	Acetone		Ethanol	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>P. mirabilis</i>	200	500	200	500
<i>P. vulgaris</i>	100	500	200	500
<i>S. aureus</i>	500	500	200	500
<i>Bacillus spp.</i>	100	500	200	500

infections treated with  $\beta$ -lactam antibiotics, at least under *in vivo* conditions without blood. Tannins exert their antibacterial activity by iron deprivation, hydrogen bonding or non-specific interactions with vital proteins such as enzymes (Scalbert, 1991).

Alkaloids have a wide range of pharmacological activities including antimalarial (for example, quinine), antiasthma (for example, ephedrine), anticancer (for example, homoharringtonine) (Kittakoop et al., 2014) etc., Chelerythrine is a benzophenanthridine alkaloid present in the plant *Chelidonium majus*, *Zanthoxylum clava-herculis* and *Zanthoxylum rhoifolium* which exhibits antibacterial activity against *S. aureus* and other human pathogens by the use of its potent, selective, and cell-permeable protein kinase C inhibitor (Gibbons et al., 2003; Luciana et al., 2014).

Pyrrolizidine alkaloids (PA) have toxic, deterrent and/or repellent effects on a wide range of generalist herbivores in order to reduce or prevent damage (Ober, 2003). PA mixtures affect microorganisms *in vivo* and *in vitro*, and variation exists in anti-microbial effects of different Pas (Hol et al., 2003). Alkaloids from *Sida acuta* was reported to have antibacterial activities against *S. aureus*, *Enterococcus faecalis*, *Shigella boydii*, *Shigella flexneri*, *Shigella dysenteriae*, and *E. coli* (Karou et al., 2005).

Gonzalez and Matthew (1982) reported that saponins, phenolic compounds and cardiac glycosides have been found to inhibit bacterial growth and are capable of protecting certain plants against bacterial infection. Antimicrobial sensitivity testing revealed that ethanol extract possesses the highest definite antimicrobial activity against *P. vulgaris* (15 mm) when compared to acetone extract with the highest being *S. aureus* (13.5mm). Similar results were observed by previous scientists against pathogenic bacteria (Talwar et al., 1997; Sharma et al., 2009). This probably explains the use of this plant by ancient people against some infections since past generations (Chatopadhyay et al., 2009). The high antibacterial activity of ethanol may be due to the extraction of higher amounts of phytochemicals compared to acetone, since both solvents are known to extract phytochemicals.

An increase in pH was an outstanding effect on the activity of the extracts with ethanol extract at 8.0 having the highest antibacterial activity against *S. aureus* (29

mm) followed by acetone extract at 8.0 against *P. mirabilis* (27 mm). Both extracts at pH 2.0 and pH 8.0 varied greatly against the microorganisms which means the effect of the change is not dependent on the extract but on the microorganisms themselves. This agrees with some herbal practices which use alkaline substances as additives for infusion. However, the inhibitory effect of the acetone extract was greater with more acidic pH (2.0) values but lowered with increased pH values (8.0) for *P. mirabilis* and *P. vulgaris* at both concentrations respectively (Table 4). This agrees with Hsieh et al. (2001).

The result of MIC for both extracts against all the microorganisms were taken, and results showed acetone extracts having MIC levels as low as 100mg/ml and all MIC levels for ethanol was recorded as 200mg/ml. For both extracts, the MBC levels across all microorganisms were recorded as 500 mg/ml. Both extracts were analyzed by t-Test: Two-Sample Assuming Equal Variances using Excel Sheet 2010 to determine which was more effective and since t Stat is less than t Critical two-tail, we accept the null hypothesis which states that there is no significant difference between the two extracts but with this in mind, the ethanol extract has more antibacterial activity than its counterpart.

In calculating the zone of inhibition and checking which extract was more effective against each microorganism, ethanol extract was more effective against all test microorganisms than acetone extract.

## Conclusion

This study showed that the extracts of *A. indica* possess antibacterial activity, and thus justifying its use in traditional medicine. From the experiments carried out, the use of *A. indica* for treatment of urinary tract infections, boils, wound infections, and diarrhea are justified. The study has also shown that the extracts work well against *P. vulgaris* followed by *S. aureus*, *Bacillus spp* and finally *P. mirabilis*. Results also reveal that the activity of the extract is actively dependent on pH. With all these said it should be noted that some phytochemicals present in the extract cause grave damage to living organisms upon large dose intake (Samanta et al., 2004).

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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