American-Eurasian J. Agric. & Environ. Sci., 16 (3): 598-604, 2016
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2016.16.3.1037

# Methanolic Extract of Neem Leaf (*Azadirachta indica*) and its Antibacterial Activity Against Foodborne and Contaminated Bacteria on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

[1,2]*Hany M. Yehia*

[1]College of Food and Agriculture Sciences, King Saud University Saudi Arabia, Al-Riyadh 11451
[2]Department of Food science and Nutrition, Faculty of Home Economics, Helwan University, Cairo, Egypt

**Abstract:** To study the effect of neem extract on the growth and viable count of foodborne and contaminated bacteria and determine its active inhibitors. Different concentrations of methanolic extracts (200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) were prepared. One-hundred microliters of standardized test organisms was treated with solvents or extract. The extract was tested against Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus*, *Clostridium perfringens* and *Micrococcus luteus*) and Gram-negative bacteria (*Salmonella typhimurium, Escherichia coli)* using agar diffusion and minimum inhibitory concentration (MIC) analyses. Methanolic neem extract was highly effective at prohibiting the growth of *Listeria monocytogenes* and *Staphylococcus aureus* at a concentration of 50 mg/ml. However, methanolic neem extract, even at the highest tested concentration (200 mg/ml), did not prohibit *Bacillus cereus* and *Bacillus subtilis* growth. In addition, three bands of *Staphylococcus aureus* proteins of different molecular weights (more than 97 kDa, 50 kDa and at 18 kDa) disappeared during the neem extract and the four antibiotic treatments. Methanolic neem extract contains a photochemical substance, such as phenolic and flavonoids, that may inhibit the growth of foodborne bacteria.

**Key words:** Neem Leaves Extract · Foodborne Bacteria · Phytochemical Analysis

## INTRODUCTION

Neem leaf extract is used as an herbal remedy and as a source of many therapeutic agents in different countries. *Azadirachta indica* is used for the treatment of diabetes because of its potential anti-diabetic properties [1]. Additionally, neem leaf aqueous extract has therapeutic potential as an anti-hyperglycemic agent in IDDM and NIDDM [2]. The anti-inflammatory effect of Neem extract is less than that produced by dexamethasone [3]. The antibacterial properties of Neem leaves have also been used for controlling airborne bacterial contamination in residential properties [4, 5] Neem seeds have also been used as a traditional medicine to treat infections, particularly in the eye and ear. Administration of Neem flower extracted by alcohol has been shown to disrupt the estrous cycle in Sprague Dawley rats and cause a partial block in ovulation, suggesting that the Neem extract may be used as an antifertility agent [6]. Neem aqueous extract has also been implicated as a possible chemotherapeutic and viral agent [7]. Flavonoid and phenolic compounds are secondary metabolites produced by plants that play an important role in the defense functions of plants. Because of their importance, several environmental factors (e.g., humidity, temperature and light) and internal factors (e.g., hormones, nutrients and genetic differences) contribute to the synthesis of flavanoids and phenolic compounds [8]. Other factors, such as variety, degree of ripening, germination, processing and storage, influence the content of phenolics in plants [9]. Additionally, phenolics are responsible for the variation in the antioxidant activity of plants [10]. The aim of the present study was to determine the antibacterial activity against foodborne and contaminated bacteria; the tannin, total flavonoid and phenolic content; and the reducing power of Neem leaf extract.

**Corresponding Author:** Hany M. Yehia, College of Food and Agriculture Sciences, King Saud University Saudi Arabia, B.O. 2460 Al-Riyadh 11451. E-mail: hanyehia@ksu.edu.sa, hanyyehia43@yahoo.de.

## MATERIALS AND METHODS

Mature plants of *Azadirachta indica* were used for this study and were collected from Khartoum city (Sudan) in 2012.

**Leaf Methanolic Extract:** The completely dried leaves were coarsely powdered and 50 g was used for successive extraction in 250 ml methanol for three days with periodic shaking. Then, the extract was filtrated and the filtrate was collected. The filtered liquid extracts were subjected to rotary evaporation and subsequently concentrated under reduced pressure (in vacuum at 40°C). Then, the extracts were evaporated to dryness and stored at 4°C in an air tight bottle [11].

**Microorganism:** Gram-positive strains of *Staphylococcus aureus* ATCC 29737, *Bacillus subtilis,* ATCC 6051, *Bacillus cereus,* ATCC 14579, *Clostridium perfringens,* ATCC 13124, *Listeria monocytogenes* ATCC 19114 and *Micrococcus luteus* ATCC 10240 and Gram-negative strains of *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 10536, were obtained from the Food Microbiology Laboratory, College of Food and Agricultural Sciences, King Saud University.

**Medium:** Peptone water (Oxoid CM0009), Muller Hinton broth (Oxoid CM0405) and Muller Hinton agar (Oxoid CM0337) were used for the preparation of the inoculum, preservation and disc diffusion agar for the bacterial strains, respectively.

**Inoculum Preparation:** Each strain was inoculated in peptone medium and incubated at 37° C for 3-4 hours and the resulting bacteria were used as inoculums. A sterile cotton swab was dipped into the bacterial suspension and then rotated and pressed on the wall of the test tube to get rid of excess fluid. The surface of a Muller Hinton Agar plate was inoculated with the bacterial strain.

**Agar Well-Diffusion Method for Screening the Antibacterial Activity of Neem Methanolic Extract:** To determine the antimicrobial activity of neem methanolic extract, the agar well-diffusion method was used. Muller Hinton agar plates were swabbed using a cotton swab from an 8 hour-old broth culture of Gram-positive or Gram-negative bacteria. By using a cork borer, wells (8-mm diameter and approximately 2-cm apart) were made in each of these plates. A stock solution of each neem methanolic extract was prepared at a concentration of 1 mg/ml in methanol. Approximately 100 µl of neem methanolic extract was added into the wells and allowed to diffuse at room temperature for 2 hrs. A control well comprising methanol without plant extract was also made. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The zone of inhibition (marked as either positive (+) or negative (-)) was used as indicator for the effect of the extract against bacterial species. According to Yehia *et al*. [12], overnight cultures of the Gram-positive strains *Staphylococcus aureus, B. subtilis, B. cereus, clostridium perfringens, Listeria monocytogenes* and *Micrococcus luteus* and the Gram-negative strains *Salmonella typhimurium* and *E. coli* were suspended in Ringer's solution to a turbidity equivalent of 0.5 McFarland ($1.5 \times 10^8$ CFU/ml) and 100 µl was spread onto Mueller-Hinton agar plates.

**Determination of Minimum Inhibitory Concentration:** Minimum inhibitory concentration was defined as the lowest concentration of the compound that inhibits the growth of microorganisms [13]. The minimum inhibitory concentration values were determined in broth dilution of neem methanolic extract in Muller-Hinton broth media. Varying concentrations of methanolic extracts (100, 50, 25, 12.5, 6.25 and 3.125 mg/ml) were prepared. One-hundred microliters of standardized test organisms was treated with solvent control or extract. The total viable count of microorganisms was assessed by pouring plate method using each neem methanolic concentration. Minimum inhibitory concentration was recorded in the plates that contained bacterial growth.

**Neem Extract Preparation:** Neem leaf was dried in an oven at 50°C and prepared by blending 50 g of the dried leaf with 100 ml of methanol for 10 min. The crude extract was filtered through muslin followed by Whatman No. 1 filter paper prior to autoclaving (121°C for 15 mins) before storage at -20°C [14].

**Polyacrylamide Gel Electrophoresis (PAGE):** The preparation of isolates for SDS-PAGE and the running of the samples were performed as follows: Electrophoresis was performed in a 12% polyacrylamide running gel and a 4% stacking gel, with a 0.025 M Tris, 0.19 M glycine buffer (pH 8.3) and 100 µL of sucrose buffer (50 mM Tris–HCl, pH 8; 40 mM EDTA, pH 8; 0.75 M sucrose).

**Preparation of Cell Extract:** An overnight culture (100 µL) was inoculated into 10 ml of fresh medium (Brain heart infusion-Oxoid, CM1135) and grown to an Optical Density

(OD) 620 of 0.6 to 0.8 (3 to 4 h). The cells were collected and weighed and 250 mg of cells was then suspended in 100 µL of TES buffer (50 mM Tris HCl, pH 8, 1 mM EDTA, 25% sucrose). Total protein was extracted from *Staphylococcus aureus* after growth on Muller-Hinton broth (Oxoid, CM0405) at 37 °C for 24 hours in the presence of neem extract (50 mg/ml) or in the presence of different antibiotics: kanamycin (30 µg), chloramphenicol (30 µg), specillin G (20 µg) and ampicillin (25 µg). Twenty microliters of lysozyme (50 mg/mL) and 5 ml of mutanolysin (5000 u/mL) were added to the suspended cells in TES buffer and incubated at 37°C for 30 min. Five to ten microliters of 20% SDS was added and the contents were mixed until they became clear. The contents were stored at -20°C for 1 to 2 days. Fifty-microliter extracts (standard and isolated bacteria) were loaded on SDS-PAGE. Electrophoresis was performed at 25°C in a vertical tank apparatus using a constant voltage power supply, until a bromophenol blue tracking dye reached the bottom of the gel. Gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad, Marnesla- Coquette, France) in water: methanol: acetic acid (6.5:2.5:1) for 18 h at room temperature. Gel destaining was performed by continuous agitation in a methanol: acetic acid: water (20:10:70 v/v/v) solvent until obvious bands of proteins were obtained. Whole-cell protein profiles of *Staphylococcus aureus* treated with neem extract or different antibiotics were examined to determine proteins that disappeared due to the treatments.

**Tannin Determination:** The aqueous extract of Neem leaf (1 ml) was mixed with 10 ml of distilled water and filtered. Three drops of Ferric chloride reagent was added to the filtrate. The presence of gallic tannins or catechol tannins was confirmed by the appearance of a blue-black or green precipitate, respectively.

**Total Phenolic Determination:** Total phenolic contents (TPC), determined by the method of Kim *et al.,* 2005 [15], were used. Four-hundred microliters of the neem methanolic extract was added to 1.0 ml of (10%) Folin-Ciocalteu reagent and mixed; then, the solution was allowed to stand at 25°C for 5 to 8 min before adding 0.8 ml of sodium carbonate solution (7.5%). Deionized water was added to a final volume of 10.0 ml. After two hours, the absorbance was measured at 800 nm. A calibration curve was prepared using gallic acid as a standard for TPC, which was measured as mg gallic acid equivalents (GAE) per gram of the sample (mg/g).

**Total Flavonoid Determination:** Determination of flavonoids was determined using a colorimetric method [16] as follows: 1.50 ml of deionized water was added to 0.25 ml of the Neem extract and then 90 µl of 5% sodium nitrite ($NaNO_2$). After six minutes, 180 µl of 10% $AlCl_3$ was added to the mixture and allowed to stand for another 5 min before the addition of 0.6 ml of 1 M NaOH. Deionized water was added to a final volume of 3 ml and mixed well. Absorbance was measured at 480 nm, after calibration with a blank. Querestin acid was used as the standard for total flavonoids to prepare a calibration curve and measured as milligram querestin equivalents (QE) per gram of the sample (mg/g), [12].

**Reducing Power Determination:** Reducing power was determined according to the method of Oyaizu [17]. Neem extract (2 ml) was mixed with phosphate buffer (0.2 M, pH 6.6, 2 ml) and 1% potassium ferricyanide (2 ml). The mixture was then incubated at 50°C for 20 min. The reaction was stopped by adding 10% trichloroacetic acid (2 ml) and then centrifuged at 5,000 rpm for 10 min. The upper layer of supernatant (2 ml) was mixed with distilled water (2 ml) and 0.1% FeCl3 solution (0.5 ml). The absorbance was measured at 680 nm against a blank with a spectrophotometer and ascorbic acid was used as a standard. The relation between the absorbance of the mixture and the reducing power was directly proportion. The percent reducing power was presented as ascorbic acid equivalents using a calibration curve between the absorbance of the reaction and the percent of the reducing power ability of ascorbic acid: OD = (0.0146 x [percent]) + 0.0016, R2 = 0.9999

## RESULTS AND DISCUSSION

The effect of methanolic extract of Neem leaves (*Azadirachta Indica*) against bacteria was determined by the diffusion method (shown in Fig 1). Methanolic extract of Neem exhibited high activity against *Bacillus subtilis, Bacillus cereus,* s*taphylococcus aureus* and *Listeria monocytogenes*. No effect was observed against either Gram-negative bacteria (*Salmonella typhimurium* and *E. coli*). The methanol extract of *Azadirachta indica* exhibited high activity against *Staphylococcus aureus*, pronounced activity against *Bacillus subtilis* and no effect against *E. coli* [18]. All tested bacteria showed a high degree of sensitivity to methanol extract of Neem leaves (*Azadirachta indica*); hence, the methanol extract exhibited broad spectrum antibacterial activity [10].

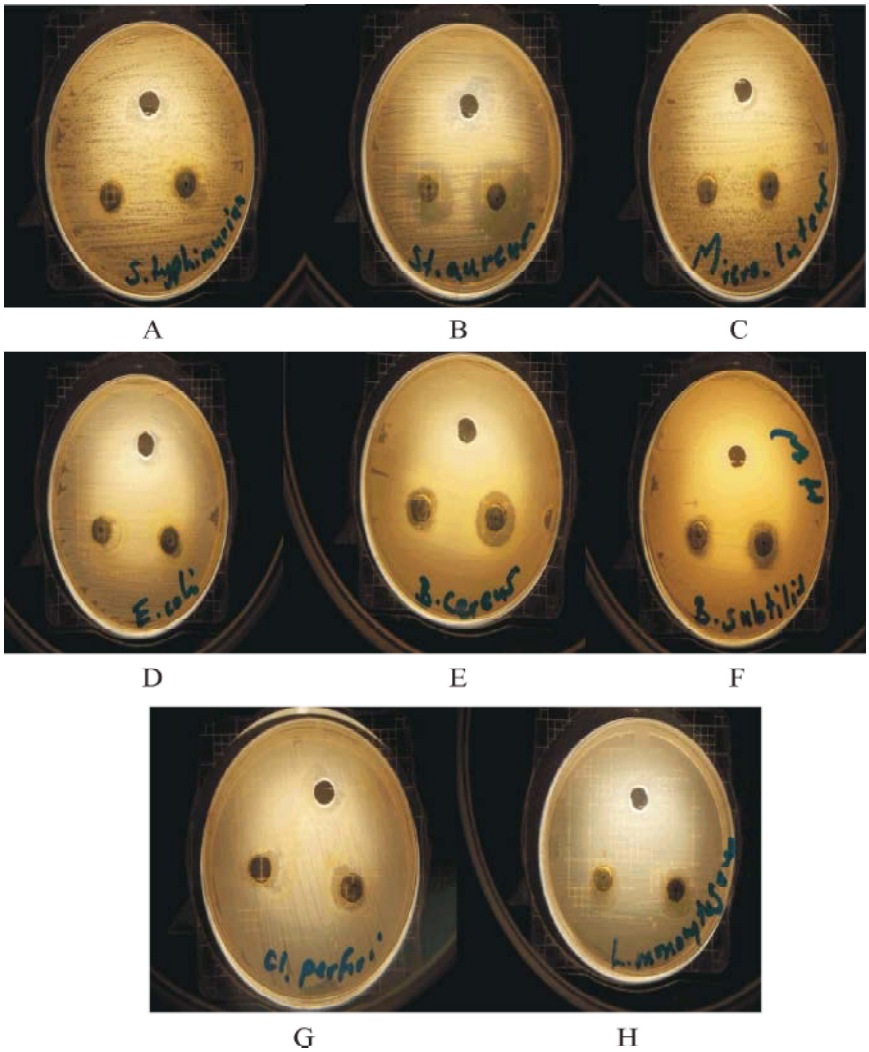


Fig. 1: Agar disc diffusion of methanolic Neem extract against different bacteria: *S. typhimurium* (A), *Staph. aureus* (B), *M. luteus* (C), *E.coli* (D), *B. cereus* (E), *B. subtilis* (F), *Cl. Perfringens* (G) and *L. monocytogenes* (H).

Increasing permeability and a greater degree of depolarization was expressed in the lipid bilayer of the Gram-negative bacteria in response to this cembranoid because the Gram-negative bacteria contain more lipids in their cell walls [19]. This depolarization effect is suggested to be associated with hydrogen bonding on the hydroxyl group in the carboxylic functionally situated at the C-19 position in the diterpene. Jente *et al.* [12], reported that chloroform and methanol produced extracts that were highly effective against *Proteus vulgaris* and *Micrococcus luteus* and to a lesser extent *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis* and *Streptococcus faecalis*. The effective components of Neem extracts are phenolic compounds, carotenoids, ketones, steroids, triterpenoids, tetratriterpenoids and azadirachtin [20]. The antibacterial activity of Neem leaf extracts (*Azadirachta indica)* might be due to presence of carotenoids, phenolic compounds, triterpenoids, ketones, valavinoids, steroids and tetra-triterpenoids azadirachtin [21].

The results in Table 1 show that the minimum inhibitory concentration (MIC) of methanolic extract of Neem leaves (*A. indica*) at a concentration of 50 and 100 mg/ml yielded the highest effect against *L. monocytogenes* and *Staphylococcus aureus* and the total viable count reduced from $1*10^6$ to 0 cfu/ml for both microorganisms. The effect of the extract against *Bacillus subtilis* was lower compared to *L. monocytogenes* and *Staphylococcus aureus*; the total viable counts changed from $1*10^6$ to $7.5*10^2$ and $6.7*10^2$ at 50 and 100 mg/ml,

Table 1: Effect of minimum inhibitory concentration of Neem methanolic extract at different concentrations on total bacterial cell count

| | Neem extract (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| Bacteria strains | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | Zone of inhibition |
| *Bacillus cereus* | 1000 | 1000 | 1000 | 1000 | 1000 | $10*10^5$ | +++ |
| *Bacillus subtilis* | $6.7*10^2$ | $7.5*10^2$ | $11.4*10^2$ | $16.8*10^3$ | $1*10^4$ | $10*10^5$ | +++ |
| *Listeria monocytogenes* | 0 | 0 | $1*10^2$ | $1*10^3$ | $1*10^5$ | $10*10^5$ | +++ |
| *Staphylococcus aureus* | 0 | 0 | $9.8*10^2$ | $1*10^3$ | $1*10^5$ | $10*10^5$ | +++ |
| *Micrococcus luteus* | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | - |
| *Salmonella typhimurium* | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | $10*10^5$ | - |
| *Escherichia coli* | $10*10^5$ | $10*10^5$ | $10*10^5$ | $-10*10^5$ | $10*10^5$ | $10*10^5$ | - |

respectively. Small effects or stable growths were shown against *Bacillus cereus* at different concentrations of the neem extract. No change in bacterial number was observed for either Gram-negative bacteria (*Salmonella typhimurium* and *E.coli*). The difference in the toxicity action against bacteria was due to the lipid bilayer composition of bacterial strains and the degree of depolarization and permeability of the cell walls [19]. Previous studies reported that this depolarization effect is associated with hydrogen bonding on the hydroxyl group in the carboxylic functionally situated at the C-19 position in the diterpene [12]. The Gram-positive non-spore-forming bacteria were more highly affected by methanolic Neem leaves extract than were the Gram-positive spore-forming bacteria due to the turnover that occurred from vegetative to spore cell. The antimicrobial potential of *Azadirachta indica* leaves and bark extract is due to its constituent flavanoids, glycosides, phyto-constituents alkaloids and saponins, which are important components of *Azadirachta indica* because they are the principles antibiotics of the plant, used as a defensive mechanism against different pathogens [22]. Neem leaves contain more alkaloid flavonoid and saponin than other parts of the plant, but Neem leaf and Neem bark contain the same phenolic percentage [22]. Bioassay-oriented fractionation of the active substances in extracts to isolate pure compounds must be performed to obtain antibiotic activities against foodborne bacterial contamination.

Figure 2 presents the effect of neem extract (50 mg/ml) and antibiotics (kanamycin (30 µg), chloramphenicol (30 µg), specillin G (20 µg) and ampicillin (25 µg)) on the total protein of *Staphylococcus aureus* after growth on Muller-Hinton broth (Oxoid, CM0405) at 37 °C for 24 hours. Three bands of proteins at different molecular weight (more than 97 kDa, 50 kDa and at 18 kDa) disappeared after the treatment of *Staphylococcus aureus* with Neem extract or the four antibiotics.

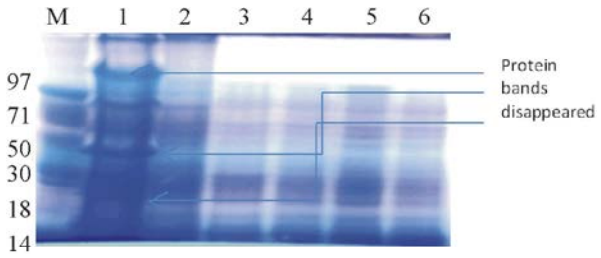


Fig. 2: M= protein marker, Whole total protein of *Staphylococcus aureus* as affected by Neem extract and different antibiotics, total extract of protein (Lane 1) as control, total extract in the presence of Neem extract (Lane2), kanamycin (Lame 3), chlorotetracycline (Lane 4), specillin (Lane 5) and ampicillin (Lane 6) on poly acrylamide gel 12% after the growth of bacteria at 37 °C for 24 hours.

The present finding revealed that the Neem leaf extract had the same effect on *Staphylococcus aureus* at that of antibiotics, as indicated by the disappearance of proteins of the same molecular weight. The effect of kanamycin on prokaryotes occurs via interaction with the 30S subunit of ribosomes, inducing a substantial amount of mistranslation and indirectly inhibiting translocation during protein synthesis [23,24]. Additionally, chloramphenicol is a bacteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. It specifically binds to A2451 and A2452 residues [19 in the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation. While chloramphenicol and the macrolide class of antibiotics both interact with ribosomes, chloramphenicol is not a macrolide. It directly interferes with substrate binding, whereas macrolides sterically block the progression of the growing peptide [25,26].

Table 2: Quantitative analysis of tannins, total phenolic content, flavonoids and reducing power in Neem extract *(Azadirachta indica)*

| Compounds | Contents (µg/ml) | Percentage (%) |
|---|---|---|
| Gallic tannin | --- | - |
| Catechol tannin | +++ | - |
| Total phenolic gallic acid | 19.375 | 0.1937 |
| Total flavanoids | 43.571 | 0.43571 |
| Reducing power | 51.945 | 0.51945 |

Baggota [27], suggested that the actions of antimicrobial agents could be adequately distinguished by certain mechanisms, such as selective inhibition of bacterial cell wall synthesis (penicillins, cephalosporins, bacitracin, vancomycin), attachment to receptors (penicillin-binding proteins) and inhibition of transpeptidation enzymes, thereby blocking the final stage of peptidoglycan synthesis (beta-lactams). This action is followed by inactivation of an inhibitor of autolytic enzymes in the bacterial cell wall.

Table 2 shows that Neem leaf extract was positive for Catechol tannins, as identified by appearance of a green precipitate, but was negative to gallic tannins. The total phenolic content of methanolic extract of neem leaves *(Azadirachta indica)* was shown in Table. 1. The results indicate that the total phenolic percentage yielded 0.1937 %. The phenolic contents of Neem leaf were 0.18 %. The percentage of total flavonoid contents was 0.43571% [28]. The Neem leaf in comparison with the other parts of the plant gave a greater percentage of flavonoid at 0.44% [28]. Flavonoid constituents are considered to be the most vital antioxidant components of herbs and a significant correlation between the concentration of plant flavonoids and the total antioxidant capacity has been reported [29-31].The reducing power of the neem leaf extract was 0.51%. There is a direct correlation between reducing power and antioxidant activity and these reductants in the Neem extract would result in the reduction of iron (III) to iron (II) [12]. The reducing power of bioactive compounds of neem leaf was related to their ability to transfer electrons, resulting into reduction. The results indicate that Neem extract contained possessed the ability to reduce iron (III) to iron (II). Variation in antioxidant activity among different medicinal plant parts is mainly due to varying amounts of phenolics, which act as reducing agents [32].

## CONCLUSION

Neem leaf extract (*Azadirachta indica*) displayed antibacterial activity against some bacteria due to the high contents of antioxidant, which confirm the presence of a bioactive compound against these bacteria. More studies focusing on Neem leaf extract in food products, such as a food additives antioxidant and food preservative against pathogenic and contaminated bacteria, must be conducted.

## ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University.

## REFERENCES

1. Shravan, K.M. and N. Kannappan, 2011. *In vivo* antidiabetic evaluation of neem leaf extract in alloxan induced rats. J. App Pharm Sci., 7:1 00-105.
2. Sonia, B. and B.P. Srinivasan, 1999. Investigation Into The Anti-Diabetic Activity of *Azadirachta indica*. Indian J. Pharmacol., 31: 138-141.
3. Abu Syed, M.D., M.D. Mosaddek and U.R. Mamun, 2008. A Comparative Study of Anti-Inflammatory Effect of Aqueous Extract of Neem Leaf and Dexamethasone Bangladesh J. Pharmacol., pp: 47-44.
4. Khan, S.A. and A. Junaid, 2008. Study on The Effect of Neem (*Azhadirachta indica*) Leaves Smoke In Controlling Airborne Bacteria In Residential Premises. Curr Res Bacteriol., (2): 64-66.
5. Mahmood, A.M., O.B. Ogbonna and M. Raji, 2010. The Antibacterial Activity of *Azadirachta indica* (Neem) Associated With Eye And Ear Infections. JMPR., 4(14): 1414-1421.
6. Gbotolorun, S.C., A.A. Osinubi, C.C. Noronha and A.O. Okanlawon, 2008. Antifertility potential of neem flower extract on adult female sprague dawley rats. Afr Health Sci., 8(3): 168-173.
7. Amer, H., A.H. Wafaa and A.A. Hanan, 2010. *In vitro* Antitumour activities of seeds and leaves Neem indica) extracts. IJAR., 2(2): 165-171.
8. Strack, D., 1997. Phenolic Metabolism. In Plant Biochemistry, P.M. Dey and J.B. Harborne, Eds, Academic Press, San Diego, pp: 387-416.
9. Bravo, L., 1998. Polyphenols: Chemistry, Dietary Sources, Metabolism and Nutritional Significanc. Nutr Rev., 56: 317-333.
10. Cai, Y., Q. Luo, M. Sun and H. Corke, 2004. Antioxidant Activity And Phenolic Compounds of 112 Traditional Chinese Medicinal Plants Associated With Anticancer. Life Sci., 74: 2157-2184.

11. Stainer, R.Y., J.I. Ingraham, M.I. Wheelis and P.R. Painter, 1986. The Relations Between Structure And Function In Prokaryotic Cells, pp: 145-182. In: P. Hall (Eds.). General Microbiology 5th Edition. Upper Saddle River, NJ.
12. Yehia, H.M., M.F. Elkhadragy and A.E. Abdel Moneim, 2011. Antimicrobial Activity of Pomegranate Rind Peel Extracts. AJMR., 4(22): 3664-3668.
13. Kumar, G.S., B.M.S. Vrushabendra, C.K.K. Ashok, K.N. Jayveera, P.U. Sanjay, R. Dhanapal and C. Sreedhar, 2007. Phytochemical and antiacne activity of Hemidesmus indicus r.br. var. Indicus Adv Pharmacol Toxicol., 8(1): 75-78.
14. Stewart, G.S., S.A. Jassim, S.P. Denyer, P. Newby, K. Linley and V.K. Dhir, 1998. The Specific And Sensitive Detection of Bacterial Pathogens Within 4 H Using Bacteriophage Amplification. J. Appl Microbiol., 84: 777-783.
15. Kim, D.O., O.K. Chun, Y.J. Kim, H.Y. Moon and C.Y. Lee, 2003. Quantification Of Polyphenolics And Their Antioxidant Capacity In Fresh Plums. J Agric Food Chem., 51: 6509-6515.
16. Dewanto, V., X. WU and R. Liu, 2002. Processed Sweet Corn Has Higher Antioxidant Activity. J. Agric Food Chem., 50: 4959-4964.
17. Oyaizu, M., 1986. Studies On Products Of Browning Reaction: Antioxidative Activities Of Products of Browning Reaction Prepared From Glucosamine. Japanese J. Nutr., 44: 307-315.
18. Bakkiyaraj, S. and S. Pandiyaraj, 2011. Evaluation of Potential Antimicrobial Activity Of Some Medicinal Plants Against Common Food-Borne Pathogenic Microorganisms. IJPBS., 2(2): 484-491.
19. Maragathavalli, S., S. Brinda, N.S. Kaviyarasi, B. Annadurai and S.K. Gangwar, 2012. Antimicrobia Activity In Leaf Extract Of Neem. IJSN., 3: 110-113.
20. Monroe, S. and R. Polk, 2000. Antimicrobial Use And Bacterial Resistance. Curr Opin Microbiol., 3(5): 496-501.
21. Koona, S. and S. Budida, 2011. Antibacterial Potential of The Extracts of The Leaves of *Azadirachta indica* Linn. Not Sci Biol., 3(1): 65-69.
22. Hafiza, R.E., 2000. Peptides Antibiotics, Lancet., 349: 418-422.
23. Pestka, S., 1975. The Use of Inhibitors In Studies on Protein Synthesis", Methods In Enzymology, 30: 261-282.
24. Misumi, M. and N. Tanaka, 1980. Mechanism of Inhibition of Translocation By Kanamycin And Viomycin: A Comparative Study With Fusidic Acid. Biochem Biophys Res Commun., 29; 92(2): 647-654.
25. Jardetzky, O., 1963. Studies on The Mechanism of Action Of Chloramphenicol-The Conformation Of Chloramphenicol In Solution" (PDF). JBC., 238(7): 2498-2508.
26. Hahn, F.E., C.L.J.R. Wisseman and H.E. Hopps, 1955. J. Bacteriol., 69(2): 215-223.
27. Baggot, J.D., 1998. Antimicrobial Selection, Administration And Dosage. JSAVA., 69(4): 174- 185.
28. Asobara, H., L.S. Joy and O.E. Amson, 2014. Comparative Study Of The Phytochemical Properties Of Jatropha Curcas And *Azadirachta indica* Plant Extracts. J PMP R., 2(2): 020-024.
29. Miller, A.L., 1996. Antioxidant Flavonoids: Structure, Function And Clinical Usage. Alt Med Rev., 1: 103-111.
30. Akdemir, Z.S., I.I. Tatli, E. Bedir and I.A. Khan, 2003. Antioxidant Flavonoids From Verbascum Salviifolium Boiss, FABAD. J. Pharm Sci., 28: 1-75.
31. Torres, R., F. Faini, B. Modak, F. Urbina, C. Labbe and J. Guerrero, 2006. Antioxidant Activity Of Coumarins and Flavonols From The Resinous Exudate Of Haplopappus Multifolius. Phytochemistry, 67: 984-987.
32. Ghimeray, A.K., C. Jin, K. Ghimire and D.H. Cho, 2009. Antioxidant Activity Quantitative Estimation of Azadirachtin And Nimbin In *Azadirachta indica* A. Juss Grown In Foothills Of Nepal. Afr J. Biotechnol., 8: 3084-3091.