International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 1; January 2018; Page No. 213-216

Antibacterial activity of chloroform extract of neem (*Azadirachta indica*) against pathogenic bacteria

Ramadass N, Subramanian N
PG and Research Department of Zoology, Arignar Anna Govt. Arts College, Cheyyar. Thiruvannamalai, Tamil Nadu, India

Abstract
Azadirachta indica known as neem has insecticide and pesticide properties all over the world. In the present study, antimicrobial activity of *Azadirachta indica* was evaluated against gram negative pathogenic bacteria (*Escherichia coli*, *Salmonella typhi a*nd *Vibrio cholerae*) and gram positive bacteria (*Bacillus subtilis*).The Chloroform leaf extracts of *Azadirachta indica* showed significant antibacterial activity against Gram positive and negative bacteria such as *Escherichia Coli, Salmonella enterica, Staphylococcus aureus* and *Enterococcus faecalis*. The in-vitro antibacterial activity was performed by agar well diffusion method. The (25, 50, 75 and 100µg/ml) leaf extract showed maximum inhibition against *Enterococcus faecalis* (29mm), *Salmonella enterica* (30mm). Phytochemical tests were performed and showed that the antibacterial activity of plant *Azadirachta indica* leaves was due to the presence of phytochemical compounds like such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponins, triterpenoids, reducing sugar, polysaccharides, phytosteroles and phenols. These works identify the specific ingredients responsible for the effect, purify it and standardize same as a drug against bacteria. The neem leaves extracts of *Azadirachta indica* showed more inhibition zone against *Escherichia Coli, Salmonella enterica, Staphylococcus aureus* and *Enterococcus faecalis.*

Keywords: chloroform, neem extract, *Azadirachta indica*, antibacterial activity

Introduction
Plants have enormous ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. A huge variety of medicinal plants and their purified components have shown beneficial including *Gymnema sylvestre, Holarrhena antidysenterica, anthelmintica, Enicostemma littorale, Momordica charantia, Swertia chirata, Azadirachta indica, Caesalpinia bonducella* and several Indian and Chinese plants. The neem *A. indica* is a common medicinal plant in India having high and wide spectrum of biological activity and well known for its insecticidal properties and one of the most promising natural compounds (Winkaler and Santos 2007) [14]. Neem is called "Arista" in Sanskrit - a word that means "perfect, complete and imperishable". This eco-friendly native tree of India is perhaps most researched tree in the world. It is a fast growing broadleaved tree, native to the arid regions of the Indian subcontinent to be found in most tropical countries (NRC, 1992) [9]. It has been in use since ancient times, to treat a number of human ailments and also as household pesticide (Chattopadhyay and Bandyopadhyay, 2005) [2]. It is renowned for its relative paucity of natural pests and pathogens, with over 300 compounds from the tree have been isolated and characterized (Kumar, 1993).The neem extract from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthosis and respiratory disorders (Ketkar and Ketkar, 1995) [6]. Every part of the neem tree has been used as traditional medicine for house-hold remedy against various human ailments from antiquity. The tree is still regarded as 'Village dispensary'. It is a plant known over 2000 years as one of the most versatile medicinal plants having a wide spectrum of activity in both developing countries (Lakshmanan and Subramanian, 1996) [8]. According to reports of the world health organization 80% of the world population relies mainly on traditional therapies which involve the use of plant extract or their active substances (WHO, 2002) [15]. The microorganism have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmad *et al*., 1998) [1]. Furthermore, antibiotics are sometimes associated with side effects (Cunha, 2001) [3], there are some advantages of using antimicrobial compounds of medicinal plants, such as fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermini and Garg, 2002) [13]. Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. Enterobacteriaceae, the enteric bacteria are facultative anaerobic Gram-Positive and negative that are live in the intestinal tract of animals in health and diseases. A number of genera within the family are human and animal intestinal pathogens (for example, *Salmonella*, *Shigella* and *Yersinia*). Several others are normal colonist of the human gastrointestinal track (for example *E. coli*, *Enterobacter*, and *Klebsiella*), but these bacteria, as well as May occasionally be associated with diseases in humans and animals (Todar, 2008) [12]. This study was planned to find out the antimicrobial activity of chloroform extracts of *A. indica*. In the present work, different extracts of neem leaves of *Azadirachta indica* were prepared using the solvents like Chloroform. The prepared extracts were screened for their anti-microbial

activity against pathogenic bacteria is done in order to detect new sources of antimicrobial agents.

Materials and Methods

Collection of plant leaf material

The leaves *Azadirachta indica* were collected from Cheyyar, Thiruvannamalai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Botany, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu. The leaves were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with various solvents.

Preparation of plant extracts

Thousand grams of dry powders leaves were taken in individual aspirator bottle; 2.5 liters of solvents (water and chloroform) were used and the mixtures were shaken approximately for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using Whatman filter paper no.1 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying. Finally the chloroform extracts of *A.indica* leaves were used for the antibacterial activity.

Antibacterial Activity

Chloroform extracts of *A. indica* were subjected for antimicrobial activity by disc diffusion method against both Gram positive and negative organism by the method of Gillespie, *et al.,* (2002).

Test organisms used

Gram positive organisms

Staphylococcus aureus (ATCC 6538)
Enterococcus faecalis (ATCC 29212)

Gram Négative Organisms

Escherichia coli (ATCC 8739)
Salmonella enterica (ATCC 10708)

Antibacterial screening test

10 and 50% solutions of chloroform extracts of leaves were prepared by dissolving 1 and 5 g in 10 ml each of distilled water. 10 ml each of the prepared concentrations were pipette into sterile test tubes. Bacterial aliquots of the test organisms were made by scooping 2 colonies each of a 24 hrs growth of the bacteria into 4 ml of sterile distilled water. 0.2 ml of each of the aliquots containing approximately 5 × 104 bacterial cells or colony forming units was transferred into both of the extract concentrations and allowed to stand for a 24 hrs for reaction to take place between the extracts and the bacterial organisms. The mixtures were then inoculated on separate nutrient agar plates and incubated at 37°C for 24hrs. Chloroform extracts of the leaves at 25, 50, 75 and 100% µg/ml different concentration were prepared and the suppressive antibacterial effect. 50% concentration of chloroform leaves extracts had inhibitory effect, hence chosen as the working concentration.

Reagents

Nutrient agar medium (Indian Pharmacopée, 1996) Beef extract (10gm) of beef extract, 10gm of peptone, 5gm of sodium chloride, 12gm of agar and 1 liter of distilled water.

Result and Discussion

As per the observations on cultured nutrient agar plates, antibacterial activity of leaves extract of neem was evaluated against both gram positive and negative bacteria. The antibacterial activity of chloroform (+) indicates presence while (–) indicates the absence of the components. Chloroform extracts was investigated using agar well diffusion method, against the Gram positive bacteria *Salmonella enterica, Escherichia Coli* and Gram negative bacteria *Staphylococcus aureus, Enterococcus faecalis*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. Table 1 and 2 showed the antibacterial activity of chloroform extracts of *A. indica* showed maximum zone of inhibition (26mm in100µg/ml) against Gram positive bacteria *Staphylococcus aureus* followed by18, 23, and 26mm in 25, 50 and75µg/ml respectively. The *Enterococcus faecalis* maximum zone of inhibition (29mm in100µg/ml) followed by 20, 24, and 28mm in 25, 50, and 75µg/ml, the another one of the Gram negative bacteria *Salmonella enterica* (30mm in100µg/ml) followed by 20, 24, and 28mm in 25, 50 and 75µg/ml, *Escherichia Coli* (22mm in100µg/ml) followed by 18, 19, and 20mm in 25, 50, and 75µg/ml. Coincide with the observations of several researchers. Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial activity action against gram-negative and gram-positive microorganisms. Saradha Jyothi Koona and Subbarao Budida (2011) [11] reported that the antimicrobial activity of the neem seed oil against a variety of pathogens. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus* spp. (Mehrotra *et al.*, 2010).

For chloroform extract, 42.56±6.24, 69.13±5.28,79.37±6.56 and 80.52±5.20 of different values of 25, 50,75 and 100 µg/ml was found to inhibit the growth concentration of *Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* at 600s (Fig.1). A comparison of the effects of commonly used antibiotics and the extracts of *A. indica* at 1, 3 and 5 mg per disc on the bacterial isolates by disc diffusion method, showed that the extracts had similar effects on the bacteria as the fluoroquinolones. The chloroformextracts of *A. indica* were able to inhibit the growth of bacterial isolates *in vitro;* study showed the plant has antibacterial properties. It is recommended that further work be done to identify the specific ingredient(s) responsible for the effect, purify it and standardize same as a drug against bacteria. The Chloroform extracts scavenging activities due to, a minimum concentration of chloroform extract, 42.25 ± 5.25, 56.68 ± 6.37, 68.99 ± 6.39 and 69.20 ± 6.14of different concentration of 25, 50, 75 and 100 µg/ml was found to inhibit the growth of *Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* at 600s(Fig.2).The results indicated that using plant parts of neem had beneficial effect in controlling the pathogenic microbial organisms and thus can be used in therapeutic formulations in near future.

Table 1: Antibacterial activity of Chloroform extract of *A.indica* in Gram positive bacteria

S. No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	*Staphylococcus aureus*	30mm	18mm	23mm	26mm	26mm	-
2	*Enterococcus faecalis*	32mm	20mm	24mm	28mm	29mm	-

Table 2: Antibacterial activity of Chloroform extract of *A.indica* in Gram negative bacteria

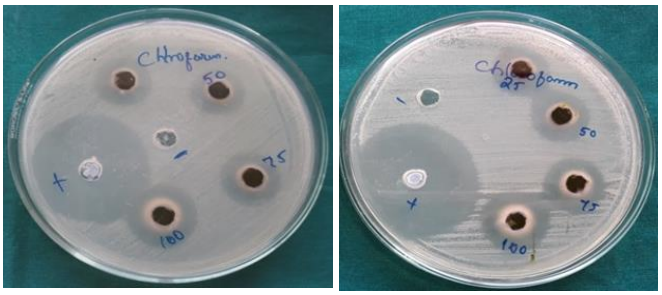
S. No	Name of the Micro organism	Reference Drug (Positive control)	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Negative Control
1	*Escherichia coli*	30mm	18mm	19mm	20mm	22mm	-
2	*Salmonella enterica*	32mm	20mm	24mm	28mm	30mm	-

Results are expressed in area of inhibition zone in mm
Reference Drug (Positive Control): Ciprofloxacin
Negative Control: DMSO
-: indicates no zone of inhibition

Table 3: DPPH radical and Nitric Oxide (NO) scavenging activity of chloroform extracts of *A.indica* leaf extract

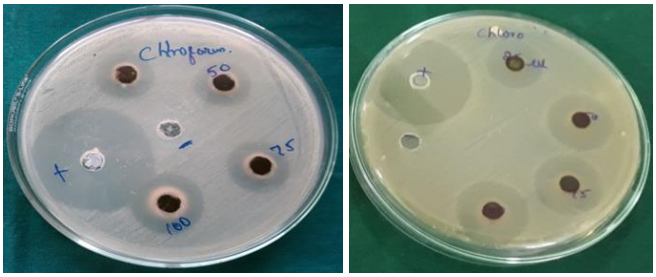
Concentration (µg /ml)	% of Inhibition	
	Chloroform extract (DPPH)	Chloroform extract (NO)
100	80.52 ± 5.20	69.20 ± 6.14
75	79.37 ± 6.56	68.99 ± 6.39
50	69.13 ± 5.28	56.68 ± 6.37
25	42.56 ± 6.24	42.25 ± 5.25

Values are expressed as mean ± SD, (n=3)



Staphylococcus aureus ***Enterococcus faecalis***

Fig 1: Antibacterial activity of chloroform extract of *A.indica* in Gram positive bacteria



Escherichia coli ***Salmonella enterica***

Fig 2: Antibacterial activity of chloroform extract of *A. indica* in Gram negative bacteria

Reference

1. Ahmad I, Method Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 1998; 62:183-193.
2. Chattopadhyay RR, Bandyopadhyay M. Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. Afr. J. Biomed. Res. 2005; 8:101-104.
3. Cunha BA. Antibiotics side effects. Med. Clin. North Am. 2001; 85:149-185.
4. Gillespie DE, Brady SF, Battermann AD, Cianciotto NP, Liles MR, Rondon MR, Clardy J, Goodman RM, Hadelsman J. Isolation of antibiotics Turbomycin A and B from a metagenomic library of soil microbial DNA Appl. Environ. Microbio. 2002; 68:4301-4306.
5. Indian Pharmoopoiea. Indian ministry of health and family welfare. Medical veterinary Michigan.isbn-65823. 1996.
6. Ketkar AY, Ketkar CM. In: Schmutterer H (ed.), The Neem tree: Source of unique natural products for integrated pest management, Medicine and other purpose. 1995; 518-525.
7. Kumar J, Naidu BA, Rao PS, Varkey M, Remany MC, Raj YCT. 2015.
8. Lakshmanan KK, Subramanian MS. *Azadirachta indica* A.S. Juss. stem-bark as anti-leprosy source. Neem and Environment, 2. Oxford and IBH Publisher New Delhi. 1996; 31143-31150.
9. National Research Council. Neem: A tree for solving global problems. National Academy Press, Washington. 1992.
10. Reena Patel, Aditi Patel, Sachin Desai, Anju Nagee. Study of secondary metabolites and antioxidants properties of leaves, stem and root among Hibiscus Rosasinensiscultivars. Asian journal of experimental biological sciences, 2012; 3(4):719-725.
11. Saradha Jyothi Koona, Subbarao Budida**.** Antibacterial potential of the extracts of the leaves of *Azadirachtaindica* Linn. Nat Sci Biol. 2011; 3(1):65-69.
12. Todar K. Pathogenic *Escherichia coli*. Online Text of Bacteriology. University of Wisconsin-Madison Department of Bacteriology. 2008.
13. Vermani K, Garg S. Herbal medicine for their sexually transmitted diseases and AIDS. J. Ethnopharmacol. 2002;

80:49-66.
14. Winkaler EU, Santos TR. Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*. Compo. Biochem. Phsiol. C. Toxicol. Pharmacol. 2007; 145(2):36-44.
15. World Health Organization. Traditional Medicine – Growing Needs and Potential. WHO Policy Perspectives on Medicine, No. 2. WHO/EDM/2002.4. WHO: Geneva. 2002.