

Original Research Article

Antibacterial activity of endemic *Artocarpus nobilis* Thw found in Sri Lanka

Abstract

Aims: To evaluate antibacterial activity of aqueous, methanol, dichloromethane, and hexane extracts of *Artocarpus nobilis* Thw. leaves and stem bark against *Escherichia coli* and *Staphylococcus aureus* using agar well diffusion method.

Methodology: Matured fully expanded leaves and stem bark parts of *Artocarpus nobilis* were collected, air-dried, and grounded. The extraction was obtained using a decoction extraction method. Antibacterial activity was performed against *Staphylococcus aureus* (ATCC® 25923TM) and *Escherichia coli* (ATCC® 25922TM) using agar well diffusion method and gentamicin was used as a positive control. The whole experiment was done in triplicates and the diameter of the inhibition zone (in mm) was measured and recorded.

Results: Results showed that aqueous bark extract (EC₅₀ 4.286 mg/mL) showed the highest efficacy and potency against *E. coli* while methanol bark extract (EC₅₀ 4.427 mg/mL) showed the highest efficacy and potency against *S. aureus*. R² and P values for aqueous, methanol, dichloromethane, and hexane extracts indicated that there was a strong, statistically significant correlation ($P \leq 0.05$) between concentration and zone of inhibition for all extracts of *A. nobilis* against *E. coli* and *S. aureus*.

Conclusion: This study showed that aqueous and methanol bark extracts of *Artocarpus nobilis* have marked *in vitro* dose-dependent antibacterial activity against *E. coli* and *S. aureus* respectively. Further studies are necessary to ascertain the mechanism and the active constituents responsible for the antibacterial activity of the of plant parts of *Artocarpus nobilis*.

Keywords: *Artocarpus nobilis* Thw., Antibacterial activity, ethanomedicine, Agar well diffusion method, Sri Lanka.

1. INTRODUCTION

There are many types of pathogenic microorganisms such as bacteria, viruses, parasites, and fungi that cause infectious diseases. Since these infectious diseases are very common among humans, implementation of preventive measures is a timely requirement [1]. The discovery of anti-microbials is the best preventive measure that was revealed in the last century. Anti-microbial agents inhibit the growth of pathogenic microorganisms by altering their pathogenic mechanisms [2]. Due to poor administration of anti-microbial drugs and irrational use of antibiotics, microorganisms have started to develop resistant mechanisms against anti-microbial agents for their survival. As a consequence, the effectiveness of anti-

microbial agents towards these pathogens got reduced. At the same time this has become a global health issue, scientists have started to investigate and evaluate the effectiveness of many medicinal plants to discover new anti-microbial agents [3 - 8].

Plants are known to be rich sources of anti-microbial agents since ancient time [9]. In most of the studies, it has proven that compounds derived from various parts of the plants have a promising therapeutic effect mainly due to the phytochemistry of the tree [10]. These compounds are very beneficial in the treatment of infectious diseases [11]. Out of many medicinal plants, *Artocarpus nobilis* Thw. was selected for the current study. *Artocarpus nobilis* belongs to the family *Moraceae* with 40 genera and 60 species [12, 41]. It is endemic to Sri Lanka and it is economically and medicinally significant tree [13, 18]. *Artocarpus nobilis* Thw. is often found in the wet zone, mid-country homesteads, and wet zone of Sri Lanka [14]. It is known in Sinhala as "Wal Del," "Badi Del," in English as "Ceylon wild breadfruit" and in Tamil as "Aresini-pilaka", "Asiri-pillakai" [15]. It is well-known for its anti-helminthic and antibacterial activities. *Artocarpus nobilis* also used in folk and ayurvedic medicine in Sri Lanka. Edible fruits and seeds are high in nutrients. Bark is used for dysentery and muscle strain, bark and latex combinations used for abscesses and blisters. According to the literature *A. nobilis* is a rich source of anti-microbial and anti-inflammatory compounds but most of these properties have not been scientifically explored. Previous studies on the phytochemistry of the genus *Artocarpus* has led to identification of many compounds like flavonoids, geranyl chalcone derivatives, geranylated phenolic constituents, stilbene derivatives, xanthenes, triterpenes and cycloartane-type triterpenoids carbohydrates, protein, fibre, different ions, vitamin C, thiamine, riboflavin, niacin, vitamin A, lutein and β -carotene etc. [16]. According to the folk and Ayurvedic literature texture of soil, amount of rain, the average temperature of a particular area would alter the quality and other aspects of a tree have been mentioned [17]. Therefore, Sri Lankan varieties of *A. nobilis* may also have variations in therapeutic properties, quality, and quantity of phytoconstituents according to the area where the plant is grown. In addition to phytochemistry, pharmacological properties such as anti-inflammatory, radical scavenging, antioxidant activity, fungicidal ability [18, 19, 20, 7, 8] and acetylcholinesterase inhibitory activity have also been investigated earlier [21].

Artocarpus nobilis has many anti-microbial properties and this study mainly focused on its antibacterial activity against intestinal infections and wound infections. Enteric (intestinal) infection is a very common condition that refers to the infection in Gastrointestinal system caused by microorganisms such as viruses (Norovirus, Rotavirus), bacteria (*E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium*), and parasites (*Giardia*, *Entamoeba*, *Ascaris*) [22]. These diseases most frequently result from consuming contaminated food or water and some can spread from person to person. Most of the time infections of the intestines result in diarrhoea or dysentery, nausea, vomiting, and abdominal cramping [23]. According to the World Health Organization [24] *Escherichia coli*, is the commonest causative agent of moderate to severe diarrhoea and food poisoning in developing and low-income countries. *Escherichia coli* is a gram-negative bacterium and they are mammalian intestinal commensals as well as pathogens [25]. Based on the above information, *Escherichia coli* strain was used to investigate the antibacterial effect of *A. nobilis* against gastrointestinal infections. When considering the wound infection, it is one of the most common hospital-acquired infections. The International Wound Infection Institute [26] defines wound infection as 'the invasion of a wound by proliferating microorganisms to a level that causes a local and/or systemic response in the host. In almost all cases wounds are colonized with potential pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta-haemolytic *Streptococci* [27]. Among these pathogens *S. aureus* is the most commonly identified pathogen responsible for wound abscesses, skin, and soft tissue infections [28]. Accordingly, to evaluate the antibacterial on wound infections, this study was

selected *Staphylococcus aureus*. *In-vitro* antibacterial activity against these two organisms was evaluated using agar well diffusion method.

2. METHODOLOGY

2.1 Plant material collection, identification, and authentication

Well grown and fully expanded fresh leaves and bark of *Artocarpus nobilis* Thw. (Bedi del / Wal del) about 500g of each were collected at flowering season from an estate in Gampaha district in Western Province, Sri Lanka (Latitude of 7° 23' 59.99" N and Longitude of 79° 98' 59.99"). Plants which were collected were identified and authenticated by a Botanist at National Herbarium, Peradeniya, Sri Lanka. Organoleptic properties such as colour, odour, and morphological characteristics such as appearance, shape, size of the *Artocarpus nobilis* Thw. (Bedi del / Wal del) were studied carefully before collection of above plant material.

2.2 Preparation of aqueous, methanol, dichloromethane, and hexane extracts of *Artocarpus nobilis* Thw. (Bedi del / Wal del) leaves and bark

Using running tap water, selected plant materials were thoroughly washed and air dried until a constant weight was obtained. The dried leaves and bark of *Artocarpus nobilis* Thw. were grounded using a grinder to obtain a fine powder material. For the extraction process, well dried and blended powder samples of each plant material were taken. The extraction was obtained using a decoction extraction method. Using an electronic balance 50 g of the fine powder was weighed and added to 500 ml of solvent measured using a measuring cylinder. This was added to the decoction apparatus then it was boiled slowly for 4 hours. The prepared extract was left for cooling and then was concentrated from the rotary vacuum evaporator into sterile glass vials. The final concentrated solid mass of the extracts was stored at 4°C in the freezer compartment of the refrigerator until used for the experiment.

2.3 Preparation of plant extracts standard concentration and positive control

Serial dilutions yielding concentrations of 1.25, 2.5, 5, 10, 20, 40, 60, 80 and 100 mg/mL were prepared from the concentrated extracts (aqueous, methanol, dichloromethane, and hexane) [29]. Gentamicin was used as the positive control of the study. It was prepared by using commercially available IV Gentamicin (80 mg/2 ml) vial. 20 mg/ml of Gentamicin solution was prepared as the final concentration of the positive control.

Comment [IM1]: Please briefly explain "Aqueous" preparation

2.4 Anti-bacterial activity screening using Agar well diffusion method

Antibacterial activity was performed against gram positive *Staphylococcus aureus* (ATCC® 25923TM) and gram-negative *Escherichia coli* (ATCC® 25922TM) bacteria by using agar well diffusion method [30, 23]. Bacterial suspension was prepared according to the 0.5 McFarland turbidity [31]. Each of the test organisms were inoculated and spread on the separate Mueller Hinton Agar (MHA) plates by using sterile swabs to obtain a confluent growth [2, 30]. After that sterile stainless-steel cylinders were used to make the wells of 6 mm in diameter, 2.5mm deep and 2 cm apart from the wall and each well on the plate[3]. Then Using the sterile inoculation needle, wells were opened and sealed the wells with sterile, melted MHA agar 10µl. Then using micropipette, 100 µL of each diluted extract

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solution was added into opened wells [32]. Gentamicin was used as positive control [33]. Sterile DMSO was used as negative control [34]. Plates were incubated for 24 hours at 37 °C. After overnight incubation, each of the inhibition zone diameters for the extracts against *Staphylococcus aureus* and *Escherichia coli* were measured and recorded in millimeters. The whole experiment was done in triplicates [3].

3. RESULTS AND DISCUSSION

Reports on anti-bacterial activity of *A. nobilis* are limited and many of these studies have conducted on the phytochemistry and few just to find out the zone of inhibition against bacterial pathogens. However, these studies have not investigated the efficacy, potency, and dose response relationship. Current study investigated *in vitro* antibacterial activity and dose response of aqueous, methanol, dichloromethane and hexane extracts of leaves and bark of *A. nobilis* which is only found in Sri Lanka.

Comment [IM4]: Image/picture

According to the results obtained, dichloromethane bark extract of *A. nobilis* showed potent antibacterial activity against *E.coli* at concentration of 100 mg/ml with an inhibition zone diameter of 22.33 ± 0.33 mm. On the other hand, methanol bark extract of *A. nobilis* showed potent antibacterial activity against *S. aureus* at concentration of 100 mg/ml with an inhibition zone diameter of 25.66 ± 0.33 mm. As shown in table 1, EC_{50} values of leaves extracts against *E. coli* as follows. Hexane (4.398 mg/mL) > methanol (4.608 mg/mL) > aqueous (8.50 mg/mL) > dichloromethane (29.96 mg/mL). Antibacterial activity of bark extracts against *E. coli* aqueous (4.286 mg/mL) > methanol (4.976 mg/mL) > dichloromethane (5.959 mg/mL) > hexane (8.95 mg/mL). When comparing both leaves and bark extracts, aqueous bark extract (4.286 mg/mL) showed the highest potency. According to table 2, Antibacterial activity of leaves extracts against *S. aureus* methanol (6.101mg/mL) > aqueous (13.26 mg/mL) > dichloromethane (13.5 mg/mL) > hexane (29.36 mg/mL). Antibacterial activity of bark extracts against *S. aureus* methanol (4.427 mg/mL) > dichloromethane (5.373 mg/mL) > hexane (32.92 mg/mL) > aqueous (65.71 mg/mL). When comparing both leaves and bark extracts, methanol bark extract (4.286 mg/mL) showed the highest potency.

Comment [IM5]: definition and interpretation

According to results in table (1) and dose-response curve (Figure 1), aqueous bark extract has the highest antibacterial efficacy and aqueous leaves extract has the lowest antibacterial efficacy against *E. coli*. On the other hand, results in table (2) and dose-response curve (Figure 2) shows, methanol bark extract has the highest antibacterial efficacy and hexane bark extract has the lowest antibacterial efficacy against *S. aureus*. According to the R^2 and P values shown in table (1, 2) and (1, 2) there was a strong positive statistically significant correlation ($P = 0.05$) between concentration and zone of inhibition by all the extracts of plant *A. nobilis* against *E. coli* and *S. aureus*. P values and R^2 values were obtained using Graphpad Prism 8 (version 8.2.1).

According to the literature of folk and ayurvedic medicine, *A. nobilis* has antibacterial activity against gastrointestinal infections as well as it is used to treat wounds and blisters. To evaluate these properties, Gram-negative *E. coli* (ATCC 25922) and gram-positive *S. aureus* (ATCC 25923) were selected respectively. Antibacterial activity was evaluated using agar well diffusion method [35, 24]. Generally, for antibacterial investigations of plant extracts methanol and aqueous extracts are considered as the best. Therefore, we have used aqueous and methanol extracts along with dichloromethane and hexane because solubility of active compounds differs with the solvent polarity [36]. The presence of phytoconstituents and secondary metabolites in the extracts, the method used for the extraction (decoction method) and the types of solvents used for the extraction process might contribute immensely for obtaining this positive antibacterial activity against *E. coli* and *S. aureus*. Extracts were rich in phytochemicals which regulate the antibacterial activity including

xanthone, xanthoangelol, terpenoids, stilbene, phenols, flavonoids, and flavone [37, 11-15]. Flavonoids act against bacteria utilizing several mechanisms. Inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of the porin on the cell membrane and inhibition of the porin on the cell membrane are some well-known examples [38]. Xanthenes and xanthone derivatives can serve as antibacterial and antioxidants by disrupting bacterial cell membranes. It induces the release of lipoteichoic acid (LTA) from the cell wall of Gram-positive bacteria, which are covalently bonded to the outside of peptidoglycan that is used in bacterial cell division and osmotic defense [39] while stilbene compounds exerts its antibacterial activity by interfering with the membrane stability and permeability of the bacterial cells [40]. At the same time, it is important to mention that there is a new emerging area of research where bacterial chaperones have been used as a novel antimicrobial target and development of novel anti microbials. Hence it is important to investigate the effect of these compounds against chaperone activity as well. Anti-inflammatory activity by plant extracts may be mediated through possible anti-bacterial activity, presence of anti-bacterial activity as shown in this study may be taken as evidence of possible anti-inflammatory activity [41, 42,43,44].

Table 1. Dose-response curve details for methanol, dichloromethane, and hexane leaves and bark extract samples of *A. nobilis* Thw plant parts against *E. coli*.

<i>Escherichia coli</i>	Aqueous extracts		Methanol extracts		Dichloromethane extracts		Hexane extracts	
	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves
EC50 (mg/mL)	4.286	8.50	4.976	4.608	5.959	29.96	8.95	4.398
R-squared	0.9495	0.9774	0.9552	0.9735	0.9758	0.7896	0.9471	0.9456
P value	0.0113	0.0055	0.009	0.0020	0.0040	0.0002	0.0015	0.007

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Table 2. Dose-response curve details for methanol, dichloromethane, and hexane leaves and bark extract samples of *A. nobilis* Thw plant parts against *S. aureus*.

<i>Staphylococcus aureus</i>	Aqueous extracts		Methanol extracts		Dichloromethane extracts		Hexane extracts	
	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves
EC50 (mg/mL)	65.71	13.26	4.427	6.101	5.373	13.5	32.92	29.36

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R-squared	0.6425	0.9448	0.9552	0.9735	0.9673	0.8603	0.9975	0.8133
P value	0.0001	0.0019	0.0097	0.002	0.0041	0.0002	0.0016	0.007

Fig. 1.: Dose-response curve for different extracts of *A. nobilis* plant parts against *E. coli*.

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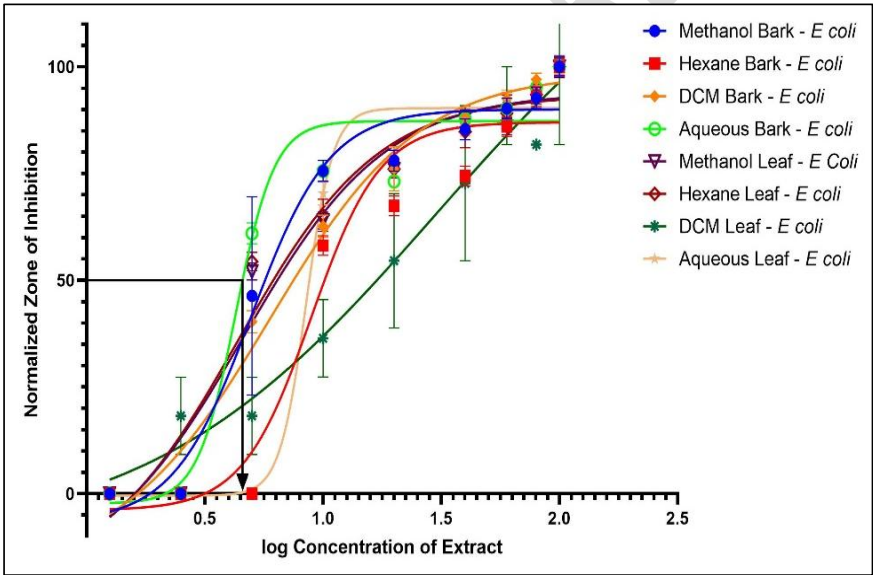
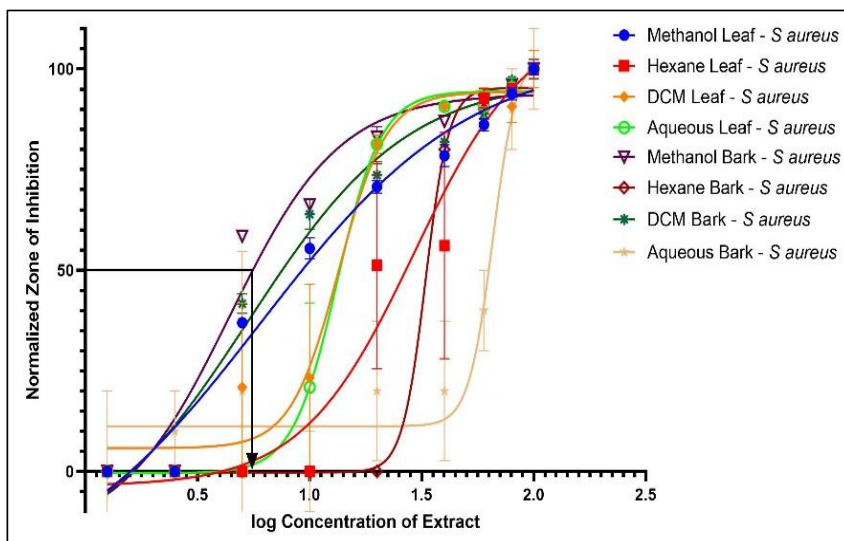


Fig. 2.: Dose-response curve for different extracts of *A. nobilis* plant parts against



S. aureus.

These graphs was prepared using Graphpad Prism 8 (version 8.2.1), using non-linear regression model according to the equation: $Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{((\text{Log IC}_{50} - X) * \text{HillSlope})}}$, where X is the log of dose response or concentration. Y= is response

4. CONCLUSION

In conclusion, this study demonstrates, for the first time, potent in vitro antibacterial activity aqueous, methanol, dichloromethane, hexane extracts of leaves and bark of *Artocarpus nobilis* against *Escherichia coli* and *Staphylococcus aureus*. The aqueous bark extract showed marked antibacterial activity *E. coli* while methanol bark showed marked antibacterial activity against *S. aureus*. Further studies are necessary to determine the mechanism and active constituents responsible for the antibacterial activity of *A. nobilis*. Moreover, our result indicates a strong possibility of developing safe potent and cheap antibacterial agent from *A. nobilis* bark.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE

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PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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