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Evaluating the Antibacterial Activities of Selected Plant Extracts in Different Solvents

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Abstract – *Several species of bacteria are responsible for the most food born deteriorate. Among them Bacillus spp. Escherichia coli and Micrococcus are the major causal organisms. Synthetic bactericide is one of most effective controlling method with health hazard. Hence, there is an urgent need for developing a bio safe method to control food borne bacterial pathogens. The aim of the present study was to investigate the Antibacterial effect of Adhathoda vasica - leaves, Azadirachta indica - seeds, Ricinus communis - seeds, Clerodendrum infortunatum-leaves and Pistia stratiotes- leaves via in vitro testing. Plants were extracted in five different solvents; methanol, ethanol, chloroform, petroleum ether and sterilized distilled water. Disk diffusion assay was conducted to evaluate the performances of each plant extract against Bacteria. A wide range of the yields among extracts was observed based on the solvent and the plant material. Ethanol plant extracts were performed best, having highest inhibition zones between 10 - 14 mm including the control with solvent only. Chloroform and Methanol plant extracts were resulted with moderate inhibition zones, while distilled water and petroleum ether were least effective against all bacterial species. The highest inhibition zone of 13.66±1.52 mm observed by Micrococcus applied in ethanolic Ricinus communis plant extract. Adhathoda vasica with ethanol was found most effective against all three tested organisms, where 12.41±2.00 mm in zone of inhibition observed in Escherichia coli cultured in ethanolic extracted Clerodendrum infortunatum while zone of inhibition was observed as 12.00±1.00 mm bacteria growth of Bacillus species. The study revealed that there is a possibility of using these plant extracts on controlling food borne diseases.*

Key words: *antibacterial effects, plant extracts, solvents, zone of inhibition*

1. INTRODUCTION

Life without nature is impossible for human beings. Food, clothes, and shelter are three basic needs of human beings, and health is the most important need, which is provided by the plant kingdom through a healthy food supply (World Health Organization 1992). Public and health are concerned with microbiological safety and quality of food due to the emergence and reemergence of foodborne pathogens across the globe (Odeyemi and Bamidele 2016; Odeyemi and Sani 2016). More than 250 sources of foodborne diseases have been identified worldwide (Scallan et al. 2015). Foodborne diseases are among over 13 zoonoses associated with over 2 billion illnesses worldwide, and more than 2 million deaths are recorded every year because of them in developing countries (Kelly et al. 2014).

Food borne diseases, caused by various bacteria, *bacillus spp. Escherichia coli* and *Microccous spp affect* agricultural food productions. Bacteria and viruses are typically the cause of food borne illnesses. Generally harmful bacteria may be already present in foods when you purchase them. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk, dairy products and fresh produce often contain bacteria that cause food borne illnesses (Todd, 2014). Traditionally, Organic acid and food preservatives are used to control food borne disease and extend the shelf life of processed food. Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit food borne bacteria in the food industry. Plants, herbs, and spices naturally occurring as antimicrobial compounds can serve as a source for antimicrobial agents against food pathogens (Deans and Ritchie 1987; Janssen et al. 1985).

Extracts containing different classes of phenolic compounds from many plants have recently gained popularity as well as scientific interest for their antibacterial and antifungal activity; Verástegui et al., 2008; Santas et al., 2010, Rauha et al., 2000; Al-Zoreky., 2009). Phenolic compounds are one of the rich sources of biocides and preservatives explored by scientists for a long time as postharvest alternative control (Lattanzio., 2003). The components such as carvacrol, eugenol, and thymol with phenolic structures, were highly active against the plant pathogens. Sri Lanka, with its rich biodiversity, is blessed with many unexplored wild herbaceous species with different capacities and which is possible to incorporate into crop development by means of fertilizers or as pesticides. They are interesting from an ethno-botanical point of view, since a lot of them are used in Sri Lanka as source of drugs in traditional and Ayurveda medicine. Furthermore, they are well known as a rich source of anti-inflammatory, diuretic, antioxidant, antibacterial, and antiviral active substances, with cosmetic as well as medicinal values (Yukawa et al., 1996; Dhiman and Chawla., 2005; Wang., 2006; Wang and Lobstein 2006).

The literature claims very low number of explorations of antimicrobial activity of phenolic extracts obtained from wild species against foodborne pathogens. Therefore, the objective of the present study was to evaluate the *in vitro* antibacterial activity of different solvent extracts of five medicinal plants. Preliminary data were analyzed to study the efficacy of the different solvent extracts of selected plants in preventing the growth of three bacterial species.

2 METHODOLOGY

2.1 Plant Materials

Abandonly available five plant species (*Pistia stratiotes, Adhathoda vasica, Ricinus communis, Clerodendrum infortunatum, Azadirachta indica*) were collected from Low Country Wet Zone in Sri Lanka and classified according to botanical and family names (Table 1). Plant parts were collected as given in the table 1 for extraction. Fresh plant parts were labeled and transported to the laboratory in sealed bags. They were cleaned and washed with distilled water followed by washing with 5% of Sodium hypochlorite (NaOCl) added with few drops of Tween-20 were used as a disinfection.

Table 1: Details about the plants used for the study

Scientific name	Common name	Plant Part used

Pistia stratiotes	Water lettuce	Leaves
Adhathodavasica	Adathoda	Leaves
Ricinus communis	Castor plant	Seeds
Clerodendruminfortunatum	Hill glory bower	Leaves
Azadirachta indica	Margosa	Seeds

2.2 Preparation of plant Crude Extracts

Analytical grade solvents; methanol, petroleum ether, ethanol, chloroform and the sterilized distilled water were used as extraction solvents. Plant tissues were homogenized by following the method described by Gurjar et al., in 2012 with slight modifications. Plant materials were grounded by using sterile mortar and pestle by adding sample: solvent as 1:10. Finely ground plant materials were subjected to shaking at 100 rpm for 24 hours at room temperature. Extracts were filtered three times with What-man 42 filter papers, by adding relevant solvent on each time followed by centrifugation at 4000 rpm for 20 minutes. Filtrate was concentrated through rotary evaporator until a sticky dark green crude extract was obtained at 700 ppm pressure and 50 °C for methanol, ethanol, petroleum ether and chloroform and at 0°C for distilled water. The crude extracts were stored in an airtight container at 4◦C until further use.

2.3 Preparation of the bacterial cultures

The pure bacterial Cultures of *Escherichia coli, Micrococus species* and Basilus *species* were collected from The Department of Botany, Faculty of Natural science, The Open University of Sri Lanka, Nawala, Nugegoda. The bacteria cultures were sub cultured from Mueller-Hinton Agar (MHA HiMedia) in Disk diffusion assay**.** Tryptic Soy Broth (TSB) media was prepared by following the manufacturer description for liquid bacterial inoculum cultures aiming rapid growth of bacteria cells.

2.4 Antibacterial assay

Antibacterial activates were performed through Disc Diffusion method by following the method explained by Mahesh and Satish (2008) with slight modifications. The pure bacterial Cultures of *Escherichia coli, Micrococcus species* and *Bacillus* species were sub cultured by streaking and inoculating into prepared Muller Hinton Agar (MHA) medium and incubated at 30 °C overnight for all bacterial species. After overnight incubation, well grown isolated colonies were streaked using inoculating wire by dipping in to aperture tube containing 2 ml of Tryptic Soy Broth (MHB). The broth culture was incubated at 35±2°C for 18 hours, centrifuged at 10,000 rpm for 10 minutes. Then the pellet was suspended in double normal saline (0.90% NaCl) to acquire the concentration of 10^6 CFU/ml of the cell density which was standardized by back calculation by plating 10^2 and 10^1CFU/ml. A bacteria cultures, which has been adjusted to 0.5 McFarland standard, were used to lawn Muller Hinton agar plates evenly using a sterile spreader. Then plates were dried for 15 minutes and then used for the sensitivity test. After solidification the filter paper discs with 5.5 mm in diameter sterilized by Autoclaving at 121°C for 20 minutes in

1.05 Kg/cm^3 finally oven dried at 60 ^{o}C for overnight were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were no closer than 24 mm from each other, center to center. Then impregnated with the 10 µl (100 µg/ml) extracts. The plates were inverted and placed in an incubator setting the temperature to 35°C within 15 minutes after the discs were applied. Plates were then incubated for 24 h at 37°C as described by the Salie et al. (1996) and Baris et al. (2006). After incubation each plate was examined. Diameter of zone of inhibition was measured to the nearest whole millimeter at the point wherein there is a prominent reduction of 80% growth. Solvents were used as negative controls. All assays were carried out in triplicate.

2.5 Data and statistical analysis

Microsoft excel software (version 13) was used for basic descriptive statistical analysis. Linear growths (LG) for antibacterial activities were calculated through measuring the inhibition zones diameter in millimeters. Antibacterial activities were measured by formula described by Mahmood *et al*., 2012. Data was analyzed by one way ANOVA by using GraphPad Prism 6.0 (Solvusoft Corporation, Las Vegas, NV, USA**)** at 95% confident level ($P < 0.05$).

3.0 RESULTS AND DISCUSSION

Escherichia coli, Micrococcus and *Bacillus species* were very sensitive to *Adhathoda vasica* - leaves, *Azadirachta indica*- seeds, *Ricinus communis* - seeds, *Clerodendrum infortunatum*- leaves and *Pistia stratiotes*- leaves as bacterial growth of this bacteria was inhibited or reduced when the growth media was amended with plant extracts. The result of the *in vitro* screening tested against *Escherichia coli, Micrococcus* and *Bacillus species* revealed that there was a significant difference (*p<0.005) in antibacterial effect among treatments when used different solvents; methanol, ethanol, chloroform, petroleum ether and sterilized distilled water. The inhibitory activity of plant extracts may be due to direct toxic effects exerted by the pathogens (Choudhury et al. 2018).

(a) (b) (c)

Plate 1: Inhibiting the growth of (a) *Escherichia coli, (b) Bacillus* species (c) *Micrococus* species by five different plant extracts

There was a significant difference among the antifungal effects among methanol, ethanol, chloroform, petroleum ether and sterilized distilled water solvents with five different plant extracts when inhibiting the growth of *Escherichia coli, Micrococcus* and *Bacillus species* as per the figures 01, 02 and 03. The high inhibition zones of 13.66±1.52 mm was observed against *Micrococcus* by ethanolic *Ricinus communis* extraction. *Adhathoda vasica* extracted in ethanol was found most effective against all three tested bacteria, where 12.41±2.00 mm zone of inhibition was measured against *Escherichia coli* and *Clerodendrum infortunatum* ethanolic extraction subpress the growth of *Bacillus* species by 12.00±1.00 mm. Ethanol extract showed significant effect on *in-vitro* inhibition against all the pathogens respectively. However, when compared with other plant extracts *Ricinus communis* had significantly higher inhibition for *Micrococcus.*

In the present study, the five plants extract screened with five solvents showed different effects on *Basillus* species. Ethanol extract of *Clerodendrum infortunatum* showed highest inhibition of the growth of *Basillus* species by allowing to measure inhibition zone of 12.00 mm. Ethanolic *Azadirachta india* and *Adhathoda vasica* inhibited the growth of *Basillus* by acquiring 11 mm and 10.31 mm zone of inhibitions respectively. Ethanolic *Ricinus communis* extract resulted with 7.8 mm inhibition in *Basillus* species. Only ethanol solvent had slightly high zone of inhibition (figure 01). According to the figure 01, *Clerodendrum infortunatum* showed higher inhibition of *Basillus species* than other plant extracts.

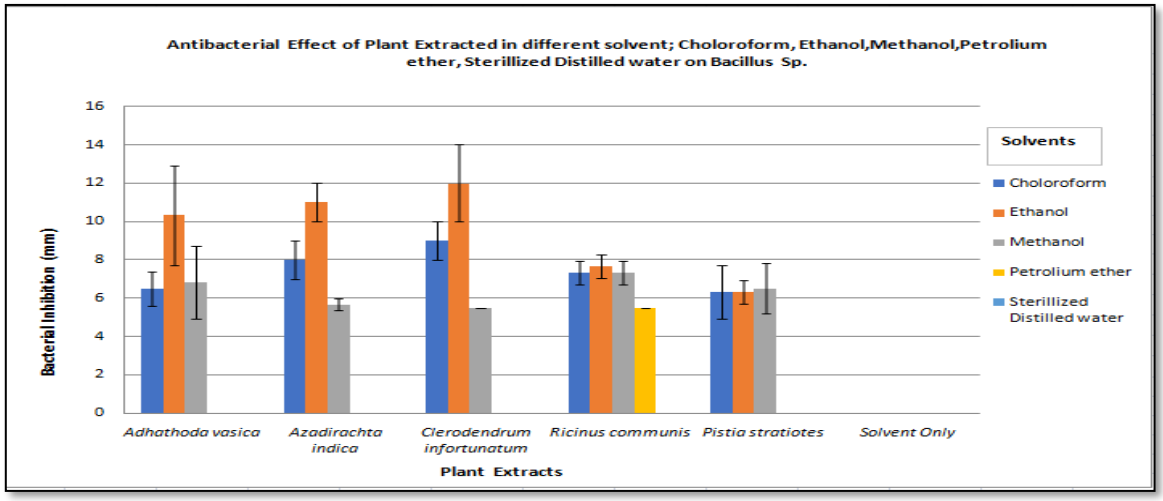

Figure 01: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Bacillus species*

Ethanolic *Adhatoda vasica* extraction resulted with 12.41 mm inhibition of *Escherichia coli* as well as Ethanolic, *Clerodendrum infortunatum* leaves, *Azadirachta indica* seeds and *Pistia stratiotes*- leaves extracts were capable gain 12 mm, 10.88 mm, 10.88 mm, inhibitory zones respectively for the growth of *Escherichia coli* (figure 02). Ethanol also gave a highest inhibition comparatively with than other four solvents. This has to be further studied to clarify the effects of the ethanol on growth inhibition on *Escherichia coli*. In addition, chloroform as well as methanol were shown higher inhibition zones in control where required further studies for clarification. Ethanolic *Ricinus communis* extracted showed significantly higher growth inhibition of *Micrococcus* than other two bacteria, beyond that, ethanolic extracted *Azadirachta indica* and Methanolic extracted *Pistia stratiotes* gave

comparatively high growth inhibition zones of the *Micrococcus* were measured as 11.51 mm and 11.44 mm.

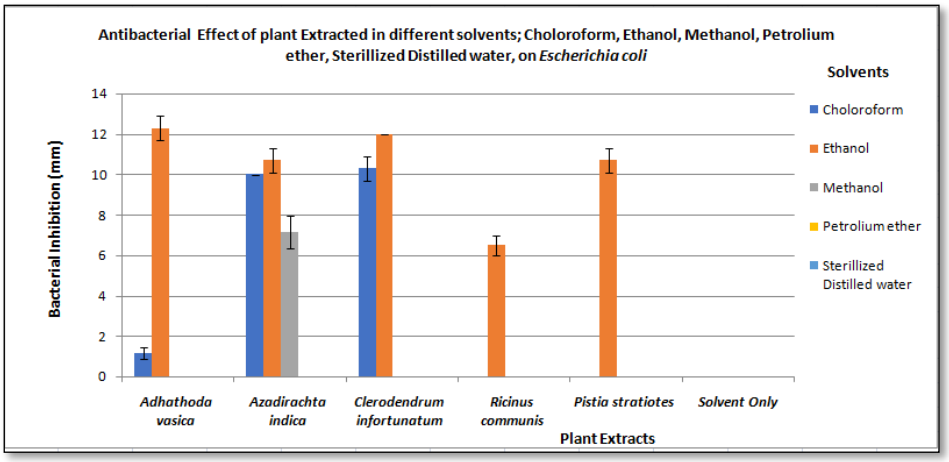

Figure 02: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Escherichia coli*

As per the findings of Hashem Rahmati et al. in *2015,* the crude extract of *Ricinus communis* seeds was found to be contained phytochemical compounds of anthocyanin, sterol, tannins and essential oils. The phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves of *Adhatoda vasica (*Karthikeyan et al. 2009). The numbers of *Clerodendrum* species were documented in ancient texts for their antimicrobial action (Neeta and Tejas 2007). Tannins are biologically active against *E. coli*, *S. aureus*, *S.paratyphi* and *C. albicans* as per the study done by Harborne et al. in 1993. Therefore, that is the reason these three plant extracts highly influence to minimize the growth of *Basillus* species, *Escherichia coli* and *Micrococcus.*

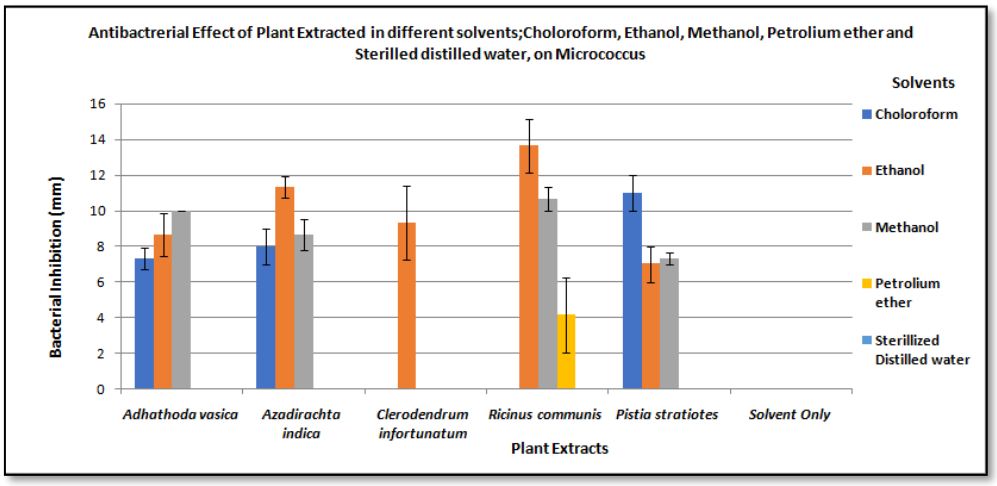

Figure 03: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Micrococcus*

Moreover, as per the figures 01, 02 and 03, with respect to the solvents used for extraction in the present study, ethanol performed best, having shown higher inhibition zone in

between 06 - 14 mm range including the plant extract with ethanol towards all three bacteria studied. Cowan, (1999) reported that ethanol and methanol can extract more active components such as alkaloids, tannins, flavonol, terpinoids, and flavones. Based on the literature and the food regulations, ethanol is known as a good solvent for certain selected food products (Alzeer and Hadeed, 2016). The antimicrobial activities of *Ricinus communis* were good against pathogenic bacterial strains *Streptococcus progenies, Staphylococcus aureus, Klebsiella pneumonia,* and *Escherichia coli* (Jena *et al*., 2012). In this study *Ricinus communis* with ethanol and methanol extracts were observed significant effect against *Micrococcus* which was high inhibiting effect of 13.66±1.52 mm diameter zone of inhibition. Uwimbabazi Francine et al., (2015) reported that Ethanol leaf extract showed higher inhibition effect than aqueous and other extract study on enable trace and witness of Neem and how it is effective on some pathogens causing diseases such as *Staphylococcus aureus* and *Escherichia coli* as experienced in the present study. The numbers of *Clerodendrum species* were documented in ancient texts for their antimicrobial action (Neeta and Tejas 2007). *Clerodendrum infortunatum* leaves extract showed effective results than root and stem extracts. The ethanol an ethyl acetate extracts were possessed a wide spectrum of antibacterial action against Gram-negative and Gram-positive bacteria (Taluar, 2014). Therefore, this study showed the highest zone of inhibition in ethanolic *Ricinus communis* – seed extracts against to bacteria *Micrococcus.*

4.0 CONCLUSION

In the present study, the natural product extracts were identified as the inhibiting agents of Escherichia *coli, Micrococcus* and *Bacillus* species with high potency. The most effective plant extracts against the three species are *Ricinus communis*, *Clerodendrum infortunatum* and *Azadirachta indica.* The preset study also confirms the *in-vitro* synergistic effect of ethanolic *Ricinus communis- seeds against the Micrococcus* resulted highest zone of inhibition. Isolation of the active compounds from ethanolic extracts could lead to improved antibacterial use in agriculture to preserve food crops as well as in the pharmaceutical industry for treatment of various bacterial diseases. Moreover, the results of the study will form the base of selection of plant species for further investigation with the potential discovery of new natural bioactive compounds.

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