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Antibacterial activity of *Nephelium lappaceum* fruit peel extracts

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ABSTRACT

Microbial safety in food products is effectively not controlled. Chemically synthesized anti-microbials that are diagnosed hazardous to human health is gradually replaced by herbal antimicrobial components. In this study the antibacterial effects of *Nephelium lappaceum* fruit peel extracts were analyzed. The efficacy of the peel extracts from *Nephelium lappaceum* were tested on two Gram negative bacteria of the strains Escherichia coli (ATCC 25922) and Salmonella typhi (MTCC 443) for the study. This comparative study was mainly aimed at using the fruit peel extract to check if they could inhibit the growth of the bacterial strains. The anti-bacterial activity was carried using Agar well diffusion method with different concentrations of Hexane and Ethanol extractions of the fruit peel.

KEYWORDS: Rambutan, Peel Extract, Antibacterial Activity

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1. INTRODUCTION

Microorganisms are widespread in nature and are used as essential tools in biology. They make up a huge part of the planets living material and plays a major role in maintaining Earth's ecosystem. Some of them is beneficial to life, but others are detrimental in nature as they can cause serious harm. Many of them are pathogens responsible for many infectious diseases in human beings that contribute a major proportion of death across the world. In order to overcome this harsh reality, the only solution is to provide medication to these diseases. Many plants have bioactive compounds (which make them) that make it as a major source of medication since time immemorial for preventing and curative purposes. Many of the fruit peels have been used as medicine from ancient days for stomach ache, sore eyes, fever etc. Malays had been using the roots and leaves of rambutan for fever and the bark for tongue infection *(Sukmandari et al., 2017, Muhamed et al., 1994).*

Rambutan, native to Malaysia, is an ever-green plant widely known in south-east Asian region as the 'king of fruits' has about 10-12cm tall and grayish brown branches. This name was derived from the Malay word 'Rambut', means hair because of the appearance of fruit with numerous hairy projections. The fruit sap is used to treat above then 50 common diseases, but there are only few studies on antimicrobial activity. As the rambutan fruit is consumed fresh, canned or processed and admired for its refreshing and exotic appearance, a huge amount of waste is produced from its peel and seed. Utilizing the rambutan waste for the production of value -added products is highly warranted as the disposed peel has been identified as a breeding site of dengue mosquitoes. Hence in the present study we tested antibacterial potency of rambutan fruit peel extracts *(Uduwela et al., 2018, Sukmandari et al., 2017, Nethgi et al., 2015).*

Rambutan peel contain mineral as Cu, Mn, Fe, Zn, Mg, K, Na, and Ca, the composition was obtained in mg/L dry rambutan peel. It has a chemical composition of fiber like cellulose, hemicellulose, lignin and has high phenolic compounds. Rambutan peel contain higher amount of polyphenolic compounds which are noticed for their biological activities. Geraniin which belongs to ellagitannins is the major compound. They are a group of hydrolysable tannins that form corilagin and gallic acid. Rambutan shows variety of biological properties due to the presences of geraniin including antioxidant, antimicrobial, anti-inflammatory, antihyperglycemic, anti-diarrheal, anesthetic and anticarcinogenic activities *(Hernández-Hernández et al., 2011, Oliveira et al., 2016, Okonogi et al., 2007).*

The methanolic extract of different cultivars of *Nephelium lappaceum* exhibited antioxidant and anti-inflammation activities which is correlated to the presences of phytochemicals such as phenolics, flavonoids, and carotenoids. It has anticancer activities through some mechanisms including cytotoxic effect, anti-proliferative and anticancer. The methanolic extract of seeds and pericarp of rambutan showed cytotoxic activities against human mouth carcinoma cells Methanolic extracts of yellow and red *Nephelium lappaceum* peels shows antimicrobial activities only in gram positive bacteria such as *Staphylococcus aureus* and not in gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. The water extract of rambutan seed also showed antibacterial activity against pathogenic bacteria such as *Staphylococcus aureus, Streptococcus pyogenes*, *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*. Ethanolic extracts of *Nephelium lappaceum* peels have exhibited anti diabetic activities on alloxan-induced diabetic male Albino rats. The

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extract also inhibited effect of carbohydrate hydrolyzing enzymes such as α glucosidase and α amylase *(Abdul Rohman, 2017).* Ethanolic extract of rambutan peel showed in vitro activity against human breast cancer cells, cervical cancer cells & osteosarcoma cancer cells and had no effect on normal cells *(Hernández-Hernández et al., 2011).*

2. MATERIALS AND METHODS

Muller Hinton agar and Muller Hinton broth were purchased from Himedia and used as per manufacturer's instructions. Sterile petridishes from Tarsons were used for the antimicrobial assay. Hexane and ethanol were purchased from Himedia. Ciprofloxacin and ampicillin from Himedia were used as the standard antibiotics in this study. Biosafety cabinet of Level 3 is used and bacterial cultures were procured from MTCC & ATCC.

2.1. Sample preparation

Nephelium lappaceum fruit peels were collected from South Vazhakulam (Ernakulam district) area to provide geographic uniformity. The fruit peels were cleaned and washed thoroughly 3-4 times in distilled water. The fruit peels were shade dried to avoid inactivation of light sensitive components if any. After proper drying which took nearly 7 weeks, the fruit peels were finely powdered and used for extraction using hexane and ethanol *(Nethaji et.al, 2015).*

2. 2. Sterilization of glass wares

All the glass wares used in the study were sterilized using the dry heat method.

2. 3. Preparation of Muller Hinton broth

Weigh 21 g of Muller Hinton broth powder and added 1 L distilled water. Heat the medium to completely dissolve the broth powder. Sterilize the medium by autoclaving at 15 lbs pressure (121°C) for 15 minutes *(Atlas, 2010).*

2. 4. Preparation of Muller Hinton Agar

Weigh 38 g of Muller Hinton agar powder and added 1 L distilled water. Heat the medium to boiling to completely dissolve the agar powder. Sterilize the medium by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The medium was mixed well before pouring into Petri dish *(Atlas, 2010).*

2.5. Maintenance of bacterial culture

The slant culture in the laboratory was revived in Muller Hinton broth overnight. From this culture another culture was initiated which lasted for 6 hours, so as to get a log phase cultureappropriate for the antibacterial assay.

2.6. Antibacterial assay

Agar well diffusion assay was employed in this study due to its simplicity and reproducibility.Before the assay all the glass wares and medium used were sterilized. Muller Hinton agar plates were prepared and the wells were dug in the petri plates using sterilized micropipette tips. The bacterial cultures were spread uniformly on the agar surface using a sterilized L shaped glass rod.

In total 4 petri dishes were used, out of which two were employed for testing the efficacy of hexane extract and ethanol extract against *Escherichia coli* (ATCC 25922) and the other two were employed for testing the efficacy of hexane extract and ethanol extract against *Salmonella typhi*. Each petridish was divided into 3 equal halves and wells were dug in the middle of two halves. First well represented negative control and the second well represented the concerned extract. Disc containing antibiotic ampicillin was placed in the middle of third half. 100 µl of each of the extracts were used at a concentration of 25mg/ml. Two separate petridishes each spread with *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) respectively were subjected to treatment with broad spectrum antibiotic ciprofloxacin. Then the inoculated petri plates were incubated at 37°C overnight. Extract having antibacterial activitywas selected based on diameter of the zone of inhibition *(Tadtong et.al, 2009, Nair et al, 2004).*

3. RESULTS

3.1. Determination of dry weight of extracts

50g of fruit peel powder was weighed and transferred to 200 ml beaker and 150 ml of hexane was added. This mixture was stirred at regular intervals and the extraction was continued till the volume reduced to approximately 10 ml. The extract were then filtered through a Whatman filter paper and collected in a petri dish and further evaporation of the solvent was allowed till a perfectly dry extract was obtained. Completely dried extract was obtained within 4 days.

The filtrate was then transferred to a new 200ml beaker and 150 ml of ethanol was added to it. The mixture was stirred at regular intervals and the process was continued till the volume reducedto approximately 10 ml. The extract was then filtered using a Whatman filter paper and further evaporation was allowed to continue in a petri dish till a complete dry extract was obtained. Complete evaporation of the ethanol extract required approximately 10 days *(Kandiah et.al, 2010).* The dry weights of each of the extract were measured using an electronic balance and the weights obtained were represented in the Table 1 below.

Table 1: Dry weight of extracts

	Name of the extract	Dry weight
1	Hexane extract	0.024
2	Ethanol extract	0.468

3.2 Antibacterial assay

Wells were dug into Muller Hinton agar plates using sterilized micropipette tips. Bacterial cultures were spread uniformly on the agar surface using sterilized L shaped glass rod. 100µl of each of the extract at a concentration of 25mg/ml was added to

the respective wells. A disc containing antibiotic ampicillin was included in each petriplate. The plates were incubated at 37°C overnight. The antibiotic ciprofloxacin was used as the positive control in this study. Antibacterial activity if present was evident as the zone of inhibition around the well *(Tadtong et.al, 2009).* The results obtained are represented in the Table 2.

Table 2: Antibacterial activity of the extracts

	Activity	Diameter of zone of inhibition
Escherichia coli		
Hexane Extract	-	Nil
Ethanol Extract	+	17 mm
Salmonella Typhi		
Hexane Extract	-	Nil
Ethanol Extract	+	13 mm

4. DISCUSSION

Plant based therapeutic remedies are gaining popularity nowadays mainly due to their relatively low side effects. The phenomenon of bacterial drug resistance that results from rampant and unscientific use of antibiotics is a looming threat in medical field. Plants with their abundant source of phytochemicals are a feasible option to rely upon as many of these bioactive compounds are blessed with antibacterial properties.

In the current study we investigated the antibacterial potency of the fruit peel extracts of the plant *Nephelium lappaceum*. Two solvent extracts prepared from the fruit peels of this plant were tested for their antibacterial activity against two gram negative bacterial strains. *Nephelium lappaceum* is an integral part of traditional medicine which is underlined by the fact that apart from the fruit, the fruit peel, leaves, roots and bark have various bioactive profiles associated with them. Rambutan is used as a remedy for diabetes, high blood pressure and thrush and also as a vermifuge and febrifuge *(Suganthi & Josephine, 2016).* As a pilot study we included two gram negative bacterial strains such as *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) for the antibacterial assay mainly due to the fact that gram negative bacteria are more prone to develop resistance against conventional therapeutic regimens.

Fruit peels of *Nephelium lappaceum*, collected from a single location (Vazhakulam in Ernakulam district) were employed in this study to provide geographical uniformity and to minimize the variation in biochemical profiles. Extreme care was taken to dry the fruit peels in the shade to prevent light inactivation of active principles, if any. Two solvents were employed for the extraction which differed considerably in their polarity such as hexane and ethanol. Ethanol extract showed maximum dry weight than hexane extract (Table 1).

Being a pilot study, we selected two gram negative bacterial strains for the antibacterial assay such as *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443). The selected bacterial strains were resistant to the antibiotic ampicillin which is used as the control for the study. The culture was revived from the slant culture maintained in the laboratory through an overnight culture in Muller Hinton broth. A second culture was initiated from the revived culture which was allowed to continue for 6 hours to obtain a log phase culture which is an absolute requirement for the antibacterial assay.

100µL of each of the extracts at a concentration of 25mg/mL were added to each of the wells dug on the Petri plates which were inoculated with the bacteria. After this the plates were incubated at 37°C for 16 hours and the zone of inhibition was measured in mm. Two negative controls were included in the study and were comprised of only hexane and ethanol. None of the negative controls elicited any antibacterial activity which demonstrated that there is no background antibacterial activity associated with the solvents used for the preparation of extracts (Figures 1 & 2). Two antibiotics, ampicillin and ciprofloxacin were included in the study. Both Escherichia coli (ATCC 25922) and Salmonella typhi (MTCC 443) used in the current study showed resistance to ampicillin. On the other hand, both Escherichia coli (ATCC 25922) and Salmonella typhi (MTCC 443) were susceptible to Ciprofloxacin which thus served as the positive control in this study.

Ethanol extract showed potent antibacterial activity against both *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) (Figure 2) and caused zone of inhibition of 17 mm and 13mm respectively. Hexane extract on the other hand did not exhibit any antibacterial activity against both *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) (Figure 1). Dry weight of the ethanol extract showed a positive correlation with the antibacterial activity which could be attributed to the increased solubility of the antibacterial constituents in ethanol. Even though both *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) showed resistance to ampicillin (Figure 1 & 2), they both were susceptible to the ciprofloxacin (Figure 3). The potent antibacterial activity displayed by ethanol extract against ampicillin resistant *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) is thoroughly encouraging and prompts future studies to include more gram negative and gram positive bacterial strains.

5. CONCLUSION

This study was deigned to evaluate the antibacterial activity of the fruit peel extracts of *Nephelium lappaceum*. Designed as a preliminary study, we employed only two solvents for the extraction procedure which differed widely in their polarity such as hexane and ethanol. Ethanol extract of fruit peels of *Nephelium lappaceum* showed significant antibacterial activity against ampicillin resistant *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443) at a concentration of 25 mg/mL Hexane extract did not elicit any activity against *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (MTCC 443). The result of the present study assumes great significance due to the very fact that the ethanol extract displayed potent antibacterial effect against ampicillin resistant bacteria and hence willdefinitely serve as a catalyst for the future studies including more gram positive and gram negative strains.

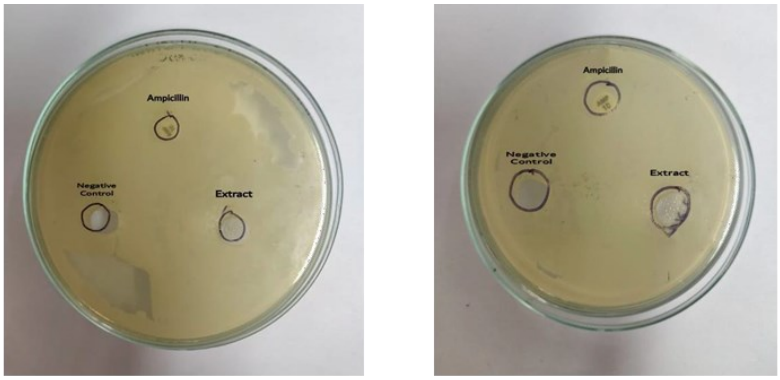

Figure 1: Effect of hexane, hexane extract and ampicillin on *Escherichia coli & Salmonella typhi*

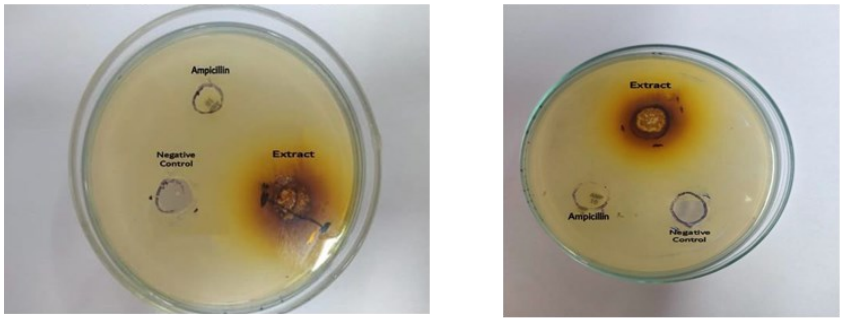

Figure 2: Effect of ethanol, ethanol extract and ampicillin on *Escherichia coli & Salmonella typhi*

Figure 3: Effect of ciprofloxacin on *Escherichia coli* & *Salmonella typhi*.

Future studies could incorporate methodologies to identify active principlesin the ethanol extracts and to extend the range of study to validate its role against fungi and viruses.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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