***Research Paper: Malini and Maheshkumar, 2013: Pp.138-142***

# EVALUATION OF BIOACTIVE POTENTIAL IN RAMBUTAN FRUIT (*Nephelium lappaceum)* SAMPLES USING PATHOGENS

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**ABSTRACT**

Antimicrobial potential of Rambutan fruit against human pathogens were screened in the present study. A pilot screening of the extracts were carried out by impregnating a 6 mm sterile Whatmann number 1 filter paper discs. The disc was loaded with the extracts to give a final load of 100,150 and 200 mg /disc. The discs were placed on the Petri plate previously seeded with the respective pathogenic strains. The inhibition – zone width was measured and calculated. The Rambutan fruit sap showed less activity against *P. aeruginosa* (6.7 ± 0.3 mm). The whole body extract (200 mg /disc) showed a high inhibitory action against *A. hydrophila* (8.1 ± 0.3 mm) and minimum effect on *S. aureus* (5.6±0.2mm). The extracts of the skin registered a maximum activity against *P. aeruginosa* (7.2 ± 0.1 mm) and minimum activity against *C. tetani* (3.6 ± 0.1). Of the three samples tested, antibacterial activity was more pronounced in fresh Rambutan fruit sap. Antifungal activity of the three samples was evaluated, it was very interesting to observe that all the above three components registered a good antifungal activity. The extracts of Rambutan fruit sap at a dose level of 200 mg/disc showed the highest inhibitory activity. A dose dependent variation of Rambutan fruit samples were observed in the antimicrobial activity. From the results Rambutan fruits have bioactive potential antimicrobial compounds.

***Key words:*** *Pathogens, Rambutan fruit sap and antimicrobial activity.*

## INTRODUCTION

Rambutan fruit is one of the most interesting of all tropical fruits widely known and revered in South East Asia as the" King of Fruits". The fruits are distinctive for its large size, unique odor and a formidable thorn covered hersk. The custard-like flesh has an exquisite flavor and is at the same time aromatic and sweet with a strange balsamic taste. Rambutan has been claimed that the fruit possess great rejuvenating power. The Rambutan fruits from the genus *Durio* belonging to the Durionaceae family. In the 1920s, Rambutan fruit products, Inc., of New York City launched a product (Dur-India) as a health food supplement selling at US$9 for a dozen bottles, each containing 63 tablets (Morton, 1987). Several medicinal investigations on the validity of this belief have been conducted with varying conclusion (Brown, 1997 and Ahmed *et al.,* 2009).

The custard-like flesh has an exquisite flavor and is at the same time aromatic and sweet with a strange balsamic taste. Rambutan has been claimed that the fruit possess great rejuvenating power (Anand *et al.,* 2007). Traditional systems of medicine, ayurveda were the basis of the health care system in India until early years of twentieth century. According to an estimation of world health organization approximately 80% of people in developing countries rely on traditional medicines for primary health care needs (Charanjit and Maini, 2001).

Rambutan fruit is one of the most interesting of all tropical fruits widely known and revered in South East Asia as the" King of Fruits". The fruits are distinctive for its large size, unique odor and a formidable thorn covered hersk (Van and Pivonka, 2000).

The presumption of world health organization is that the edibles, particularly of plant derived, empathetically indicates that these items have medicinal values (Khan and Siddiqui, 2007). The drugs are derived from the whole plant or from different organs, like leaves, stem, bark, root, flower, fruits, seed etc. Some drugs are prepared from excretory plant product such as gum, resin and latex. Not only, that plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs (Chansiripornchai , 2008). The scientific study of traditional medicines, derivation of drugs through bio prospecting and systematic conservation of the concerned medicinal plants are thus of great important. Many phytochemical found in fruits act as powerful antioxidants that give them color, flavor, odor and protection against human diseases. Rambutan fruits were used as good sex tonic and its fruit sap is very useful in treating more than 50 common diseases. But there is no scientific study on the antimicrobial activity of the extracts of the Rambutan fruits [11]. Hence in the present study attempt has been carried out to find out the antimicrobial activities of the extracts of the whole fruits, skin and fresh Rambutan fruit sap.

## MATERIAL AND METHODS

Sample of fruit of rambutan was collected from local market at Courtallam, Tirunelveli district, Tamilnadu, India and brought to the laboratory for the estimation of bioactive potential (Antibacterial and antifungal assay) using pathogenic strains.

Fruits sample was extracted with aqueous solution in 1g/ml (stock solution). The disc was loaded with the extracts from stock solution to give a final load of 100,150 and 200 mg /disc. Pathogens were collected from Arvind hospital lab, Tirunelveli, Tamilnadu, India. All the cultures were cultured in nutrient and Rose Bengal broth (Hi-media) and incubated at $37^0$/RT$^0$ C for 24 hours. Results of fruits antimicrobial activity was compared with standard antibiotic Ciprofloxacin (40µg/ml) for bacterial pathogen and Myconozole (50µg/ml) for fungal pathogens.

The human bacterial pathogens such as *Staphylococcus aureus*, *Salmonella pneumonia*, *Clostridium diphtheria*, *Bacillus cereus*, *Clostridium tetani*, *Escherichia coli*, *Salmonella typhi*, *Aeromonas hydrophila*, *Klebsiella pneumonia*, *Pseudomonas aerugenosa* and fungal pathogen such as *Aspergillus niger*, *A. fumigatus*, *Candida albicans*, *Phytophthora infestants and Trichophyton rubrum* were selected for antimicrobial screening. Ten microlitre of the broth culture was aseptically transferred to the air dried sterile agar plates and spread the culture uniformly with the help of a sterilized spreader made up of glass rod.

The extracts of skin, whole body and fresh Rambutan fruit sap were subjected to pilot study. A pilot screening of the extracts were carried out by impregnating a 6 mm sterile Whatmann number 1 filter paper discs. The disc was loaded with the extracts to give a final load of 100,150 and 200 mg /disc. The discs were allowed to dry completely and after the aqueous solvent was evaporated, the discs were placed on the Petri plate previously seeded with the respective pathogenic strains. Three replicates were used for each treatment. Control discs were kept without any extracts but soaked in respective microlitre of aqueous solvent and dried plates were then kept at $37^0$ C in an incubator for 24hrs. The inhibition – zone width (distance from the edge of the paper disc to the outer edge of the inhibition zone) was measured to the nearest mm, at 24hrs by using Hi-Media antibiotic zone scale and expressed in standard deviation of mean (± SE). The antimicrobial activity of fresh Rambutan fruit sap, the extracts of skin and whole body were calculated for different concentrations.

The minimum inhibitory concentration (MIC) of fruit extract was determined by Kuete *et al.*, method [14]. The test extract was serially diluted two fold to obtain concentration ranges 2mg/ml to 122 mg/ml for aqueous extract in titre plates. One hundred microlitres of each concentration was added in a well containing nutrient broth and 5 microlitres of inoculums, the negative control well consisted of 195 microlitres of nutrient broth and 5 microlitres of inoculums. Two hundred microlitres of nutrient broth considered as blank. The plate was covered with sterile plate scale and incubated in $37^0$C for 24 hrs. After incubation, the optical density was read at 520nm. The lowest optical density read in minimum inhibitory concentration of test fruit extract.

**RESULTS AND DISCUSSION**

Results on the antibacterial activity of various extracts of Rambutan fruits are presented in the Tables 1 –3. The Rambutan fruit sap, outer skin and whole body extracts of the Rambutan fruits showed a good antibacterial activity. Of the three samples tested, antibacterial activity was more pronounced in fresh Rambutan fruit sap. Next to Rambutan fruit sap, the whole body extracts showed a good antibacterial activity. Of the different bacteria tested antibacterial activity was well expressed against S. *typhi and C. tetani*. The Rambutan fruit sap showed less activity against *P. aeruginosa* (6.2 ± 0.1 mm). The whole body extract (200 mg /disc) showed a high inhibitory action against *A. hydrophila* (7.6 ± 0.1 mm) and minimum effect on *S.aureus* (5.0±0.1mm). The extracts of the skin registered a maximum activity against *P. aeruginosa* (6.7 ± 0.1 mm) and minimum activity against *C. tetani* (3.5 ± 0.1). In standard antibiotic, Ciprofloxacin (40µg/ml) showed maximum inhibition in all the tested bacterial pathogens from 14.3 to 26.3 mm. The antibacterial potential in the Rambutan fruit sap and whole body extracts of Rambutan fruits suggests that the mite possess antibacterial compounds and this has to be explored in future.

Antifungal activity of the extracts of whole Rambutan fruits, skin and fresh Rambutan fruit sap was evaluated. It was very interesting to observe that all the above three components registered a good antifungal activity. For the present investigation, five fungal organisms were selected and the results are presented in the Tables 4 - 6. Of the three products of fruits tested, the fresh Rambutan fruit sap registered the maximum antifungal activity. Next to the Rambutan skin fruit extract, the aqueous extracts of the whole fruit exhibited a low level antifungal activity. A dose dependent variation was observed in the antifungal activity. The extracts of Rambutan fruit sap at a dose level of 200 mg/disc showed the highest inhibitory activity. Of the five fungal species tested the dermatophyte, *Trichophyton rubrum* responded much to the extracts of the skin and the Rambutan fruit sap of the Rambutan fruits. Inhibition zone for *T. rubrum* was 8.0 ± 0.1 mm for Rambutan fruit sap and 6.8 ± 0.1 for the skin extracts. This was followed by *Phytophthora infestants*. It showed an inhibition zone of 7.5 ± 0.1 for Rambutan fruit sap and 6.7 ± 0.1 for skin extract. Antifungal Property was poor in *Aspergillus niger*. The inhibition zone for *Aspergillus niger* was 7.1± 0.1 mm for the

Rambutan fruits sap and 5.0 ± 0.1 mm for the skin extract of Rambutan fruits.

The whole body extract of Rambutan fruits had lesser antifungal activity than the Rambutan fruit sap and skin extract of fruit. Here also a dose level of 200 mg/ disc showed the highest inhibitory activity than 100 mg/disc and 150 mg/ disc. The whole body extracts showed a less inhibitory activity (3.3 ± 0.1 mm) against *T. rubrum* at a dose level 200 mg / disc. The response of *Candida albicans* was more to the whole body extract of Rambutan fruits. Inhibition zone for *C. albicans* was 6.1 ± 0.1 mm at a dose level 200mg/ disc. The antifungal activities in the extracts of the fruit skin promote the possibility to develop new drugs. In standard antibiotic, Myconozole (50µg/ml) showed maximum inhibition in all the tested fungal pathogens from 18.4 to 23.4 mm.

The degree of inhibition as measured by the disc diffusion method, reported that the gram negative bacteria were more inhibited than the gram positive bacteria. Similar findings were also reported by various workers (Haruenkit *et al.,* 2007 and Muhammad *et al.,* 2010) in different fruits. The present investigation confirmed the previous work (Komsil and Pongsamart, *et al.,* 2010) of Rambutan fruits antimicrobial activity against pathogenic organisms. The fruit extract suppress the growth pathogens. It is interesting to note that fruits may have bioactive compounds and antimicrobial activity due to the presence of various glycosides, tannins, phenols and flavonoids (Sunanta *et al.,* 2003). Therefore, present investigation may demonstrate antimicrobial activity of Rambutan fruit extracts. The present study is a preliminary attempt in evaluating the antimicrobial activity of fruit extracts. Further pharmacological investigations are warranted in this direction for establishing its detailed mechanism of action and for substantiating its traditional and folk claims.

In aqueous extracts of test fruit sap samples showed lowest minimum inhibitory concentration values against all the test pathogens compared to shin and whole fruit extracts, the ranges of minimum inhibitory concentrations 16 to 64 µg/ml were observed in all extracts of fruit (Table 7). In recent years development of multidrug resistance in the pathogenic bacteria had created major clinical problems in the treatment of infectious disease. This and other problems such as toxicity of certain antimicrobial drugs on the host tissues triggered interest in search of new antimicrobial substances/drugs of plant origin (Sunanta *et al.,* 2003).

**CONCLUSION**

In conclusion, the finding in this study suggests that the Rambutan fruits possess antimicrobial activity against pathogens. Fruits are the important source in our diet. They ate nutritious and medicinally important. Fruits are also very low in zinc which is essential for the function of the immune system, the formation of skin, the healing of wounds, brain function and it is essential for the function of the reproductive organs. But do not despair; this deficiency can be made up by eating plenty of green leaves. In the present study hope this report has given some new insights into fruit and that society will consider growing and eating more of these wonderful foods in seasonal available fruits.

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**Table 1.** Antibacterial activity of whole fruit extracts against pathogenic bacteria.

| Organisms | Name | Zone of inhibition (mm) (± S.D) Concentration of whole sample (mg/disc) | | | Standard antibiotic Ciprofloxacin |
|---|---|---|---|---|---|
| | | 100 | 150 | 200 | (40µg/ml) |
| Gram Positive Organism | *S. aureus* | 2.8 ±0.1 | 3.6 ± 0.2 | 5.1 ± 0.2 | 14.3 |
| | *S. pneuomoniae* | 3.6 ± 0.2 | 4.1 ± 0.1 | 5.7 ± 0.1 | 16.5 |
| | *C. diphtheria* | 3.6 ± 0.1 | 4.6 ± 0.1 | 5.8 ± 0.2 | 16.4 |
| | *B. cereus* | 3.7 ± 0.2 | 4.6 ± 0.1 | 6.8 ± 0.1 | 18.4 |
| Gram Negative Organism | *C. tetani* | 3.6 ± 0.1 | 5.1 ± 0.1 | 6.6 ± 0.1 | 19.3 |
| | *E. coli* | 3.5 ± 0.2 | 4.9 ± 0.2 | 5.8 ± 0.2 | 16.6 |
| | *S. typhi* | 4.0 ± 0.1 | 4.8 ± 0.1 | 5.9 ± 0.1 | 19.4 |
| | *A. hydrophila* | 4.0 ± 0.1 | 4.6± 0.1 | 7.6 ± 0.1 | 26.3 |
| | *K. pneuomoniae* | 3.6 ± 0.1 | 4.9 ± 0.1 | 7.2 ± 0.2 | 25.4 |
| | *P. aerugenosa* | 3.7 ± 0.2 | 4.6 ± 0.2 | 6.7 ± 0.2 | 24.1 |

**Table 2.** Antibacterial activity of skin extracts of fruits against pathogenic bacteria.

| Organisms | Name | Zone of inhibition (mm) (± S.D) Concentration of skin extract(mg/disc) | | | Standard antibiotics Ciprofloxacin |
|---|---|---|---|---|---|
| | | 100 | 150 | 200 | 40µg/ml |
| Gram Positive Organism | *S. aureus* | 2.7 ±0.2 | 3.7 ± 0.2 | 4.6 ± 0.2 | 14.3 |
| | *S. pneuomoniae* | 3.0 ± 0.1 | 3.7 ± 0.1 | 4.9 ± 0.1 | 16.5 |
| | *C. diptheriae* | 3.0 ± 0.1 | 3.8 ± 0.2 | 6.1 ± 0.3 | 16.4 |
| | *B. cereus* | 3.1 ± 0.2 | 2.8 ± 0.1 | 3.7 ± 0.2 | 18.4 |
| Gram Negative Organism | *C. tetani* | 3.0 ± 0.1 | 2.8 ± 0.1 | 3.1 ± 0.1 | 19.3 |
| | *E. coli* | 3.8 ± 0.2 | 3.7 ± 0.2 | 4.1 ± 0.3 | 16.6 |
| | *S. typhi* | 4.0 ± 0.1 | 5.7 ± 0.1 | 5.7 ± 0.2 | 19.4 |
| | *A. hydrophila* | 3.0 ± 0.1 | 5.0 ± 0.1 | 7.1 ± 0.3 | 26.3 |
| | *K. pneuomoniae* | 2.1 ± 0.2 | 3.2 ± 0.2 | 4.1 ± 0.2 | 25.4 |
| | *P. aerugenosa* | 3.0 ± 0.2 | 4.7 ± 0.1 | 6.7 ± 0.1 | 24.1 |

**Table 3.** Screening of antibacterial potential of fresh Rambutan fruit sap using pathogens.

| Organisms | Name | Zone of inhibition (mm) (± S.D) Concentration of hemolymph (mg/disc) | | | Standard antibiotics Ciprofloxacin |
|---|---|---|---|---|---|
| | | 100 | 150 | 200 | 40µg/ml |
| Gram Positive Organism | *S. aureus* | 4.0 ±0.2 | 4.1 ± 0.2 | 6.7 ± 0.1 | 14.3 |
| | *S. pneuomoniae* | 4.1 ± 0.2 | 5.1 ± 0.1 | 7.6 ± 0.2 | 16.5 |
| | *C. diptheriae* | 3.7 ± 0.1 | 5.2 ± 0.1 | 6.7 ± 0.1 | 16.4 |
| | *B. cereus* | 3.1 ± 0.2 | 4.7 ± 0.2 | 7.8 ± 0.2 | 18.4 |
| Gram Negative Organism | *C. tetani* | 4.7 ± 0.1 | 6.1 ± 0.1 | 8.1 ± 0.1 | 19.3 |
| | *E. coli* | 4.7 ± 0.1 | 5.7 ± 0.2 | 7.1 ± 0.2 | 16.6 |
| | *S. typhi* | 5.7 ± 0.2 | 6.1 ± 0.1 | 7.9 ± 0.2 | 19.4 |
| | *A. hydrophila* | 3.0 ± 0.1 | 5.7 ± 0.1 | 7.8 ± 0.1 | 26.3 |
| | *K. pneuomoniae* | 4.0 ± 0.1 | 4.7 ± 0.2 | 7.0 ± 0.1 | 25.4 |
| | *P. aerugenosa* | 2.9 ± 0.2 | 4.7 ± 0.1 | 6.2 ± 0.1 | 24.1 |

**Table 4.** Screening of antifungal activity of Rambutan fruit sap using fungal pathogens.

| *Organisms* | Zone of inhibition (mm) (± S.D) Concentration of Rambutan fruit sap(mg/disc) | | | Standard antifungal agent Myconozole |
|---|---|---|---|---|
| | 100 | 150 | 200 | 50µg/ml |
| *Aspergillus niger* | 4.1 ±0.1 | 4.7 ± 0.1 | 7.1 ± 0.2 | 20.3 |
| *Aspergillus fumigatus* | 4.2 ± 0.1 | 5.0 ± 0.1 | 7.6 ± 0.2 | 18.4 |
| *Candida albicans* | 4.3 ± 0.3 | 5.1 ± 0.1 | 7.0 ± 0.1 | 21.6 |
| *Phytophthora infestants* | 3.3 ± 0.2 | 5.0 ± 0.2 | 7.5 ± 0.2 | 20.5 |
| *Trichophyton rubrum* | 5.3 ± 0.1 | 6.2 ± 0.1 | 8.0 ± 0.1 | 20.2 |

**Table 5.** Antifungal activity of skin extracts of fruit against fungal organisms

| *Organisms* | Zone of inhibition (mm) (±S.D) | | | Standard antifungal agent Myconozole |
|---|---|---|---|---|
| | Concentration of skin extract(mg/disc) | | | |
| | 100 | 150 | 200 | 50µg/ml |
| *Aspergillus niger* | 3.1 ± 0.2 | 3.9 ± 0.2 | 5.0 ± 0.1 | 20.3 |
| *Aspergillus fumigatus* | 3.6 ± 0.1 | 4.1 ± 0.1 | 5.9 ± 0.1 | 18.4 |
| *Candida albicans* | 3.8 ± 0.2 | 5.0 ± 0.2 | 6.2 ± 0.1 | 21.6 |
| *Phytophthora infestants* | 4.2 ± 0.2 | 4.7 ± 0.1 | 6.8 ± 0.2 | 20.5 |
| *Trichophyton Sp* | 4.1 ± 0.1 | 4.9 ± 0.2 | 6.8 ± 0.1 | 23.4 |

**Table 6.** Antifungal activity of whole fruit extracts against fungal organisms

| *Organisms* | Zone of inhibition (mm) (± S.D) | | | Standard antifungal agent Myconozole |
|---|---|---|---|---|
| | Concentration of whole sample (mg/disc) | | | |
| | 100 | 150 | 200 | 50µg/ml |
| *Aspergillus niger* | 3.1 ± 0.2 | 4.0 ± 0.1 | 5.0 ± 0.1 | 20.3 |
| *Aspergillus fumigatus* | 3.2 ± 0.1 | 3.8 ± 0.2 | 5.2 ± 0.1 | 18.4 |
| *Candida albicans* | 3.3 ± 0.1 | 4.1 ± 0.1 | 6.1 ± 0.2 | 21.6 |
| *Phytophthora infestants* | 3.1 ± 0.2 | 3.2 ± 0.2 | 4.1 ± 0.2 | 20.5 |
| *Trichophyton rubrum* | 2.9 ± 0.1 | 3.0 ± 0.1 | 3.3 ± 0.2 | 23.4 |

**Table 7.** Minimum inhibitory concentration (MIC) of test fruit extracts against pathogens.

| Organisms | Name | Test fruit sample (µg/ml) | | |
|---|---|---|---|---|
| | | fruit sap | skin extracts | whole fruit |
| Gram Positive Organism | *S. aureus* | 16 | 64 | 32 |
| | *S. pneuomoniae* | 32 | 64 | 64 |
| | *C. diptheriae* | 16 | 32 | 32 |
| | *B. cereus* | 16 | 64 | 32 |
| Gram Negative Organism | *C. tetani* | 32 | 64 | 64 |
| | *E. coli* | 32 | 64 | 64 |
| | *S. typhi* | 32 | 64 | 64 |
| | *A. hydrophila* | 16 | 32 | 32 |
| | *K. pneuomoniae* | 32 | 64 | 64 |
| | *P. aerugenosa* | 64 | 64 | 32 |
| Fungal pathogens | *Aspergillus niger* | 32 | 64 | 64 |
| | *Aspergillus fumigatus* | 16 | 64 | 32 |
| | *Candida albicans* | 32 | 64 | 32 |
| | *Phytophthora infestants* | 16 | 64 | 32 |
| | *Trichophyton rubrum* | 16 | 32 | 16 |

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