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Antifungal Effects of Artocarpus Heterophyllus Latex and Cocos Nucifera Husk Extracts on Candida Microorganisms – A Microbiological Study

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ABSTRACT

Plants have an almost infinite ability to synthesize compounds that have diverse bioactive properties. Among the large number of plant species that have potential medicinal value, plants like *Artocarpus heterophyllus* (Jack fruit) and *Cocos nucifera* (Coconut) have been reported to possess potent biological properties to be used as antimicrobial agents. Jack fruit has extensive medicinal properties and has long history in the health care system of tropical countries. Folklore medicine claims that coconut is being used in diabetes, diarrhea and pneumonia. In view of their importance in medicinal chemistry, the present study reports the evaluation of antifungal effects of *Artocarpus heterophyllus* latex and *Cocos nucifera* (Coconut) husk on candida microorganisms using agar well diffusion method. The crude extract prepared in solvents such as acetone, di-ethyl ether and ethyl alcohol were tested separately for their fungicidal property against candida organisms. The bioassay was repeated for eleven times where the zone of inhibition was observed and measured for each test organism. Using one-way ANOVA, the mean values of all the experiments were calculated and statistically analysed. The intergroup comparisons of different inhibition zones of each solvent extracts were compared using Kruskal Wallis test. The activity results suggested that among the solvent used acetone husk extract possessed significant fungicidal property against the candida species compared to that of ethyl alcohol extract. All the statistical analyses were done using Statistical Package for Social Sciences version 16.0 and the detailed results are reported.

Keywords: Artocarpus heterophyllus; Cocos nucifera; Antifungal; Candida Species; Organic Solvents.

INTRODUCTION

It is well known fact that India is a country that possess abundant natural resources and the knowledge of traditional medicine. The use of plants as source of new drugs is an innate and very important component of healthcare system. However, very little information is available about many useful herbs in the form of experimental data. Many families of plants such as Cariaceae, Arecaceae, Moraceae, Euphorbiaceae and Apocynaceae were growing widely throughout the tropical and subtropical regions of Karnataka. Some of the plants from these families are popularly known to produce medicinally important latexes which are found to be sources of various biologically active compounds [1].

Cocos nucifera (Arecaceae family) is one such plant which is beneficial with added effect on oral health. The most interesting feature of coconut is its fruit wall. It has three layers namely exocarp, mesocarp

and endocarp. Due to extensive crosslinking between phenolics, lignin and polysaccharide, the mesocarp becomes hard and fibrous. A major portion of this waste material is carbohydrate and phenolic in nature [2]. The oil and milk derived from it are commonly used in cooking and frying. Coconut oil is also widely used in soaps and cosmetics. The clear liquid coconut water within is a refreshing drink and can be processed to create alcohol [3,4]. The exocarp and mesocarp make up the "husk" of the coconut. The coconut husk is 5-10 cm thick fibrous covering of the coconut which envelops the hard-shell structure of 3.5 mm thickness. The colour of husk is dull brown when fully ripe. The husk is full of long, coarse fibres, all running in one direction. The fibres are embedded in a matrix of material called coir dust [3,4]. The husk and shells can be used for fuel and are a source of charcoal. The husks and leaves can be used as material to make a variety of products for furnishing and decorating. The whole tree has valued place in research due to its medicinal and nutritive properties. However only few reports are available which are related to the antifungal activity of this taxon [3].

Artocarpus heterophyllus (Jackfruit) is an herbaceous plant belongs to the family Moraceae. It mainly grows in tropical areas, the Western Ghats of India and also found in Central and Eastern Africa, South – Eastern Asia, Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands. It is a large, evergreen tree with rough straight stem and green or black bark, exuding milky latex with broad elliptic leaves [5]. The leaves are found useful in treating conditions such as illness, boils, wounds and skin diseases [6]. Numerous studies have been done by various researchers to assess the phytochemical constituents of latex jack fruit. It contains a diversity of compounds especially phenolic compounds, flavonoids and tannins [7-10]. These phytonutrients have a wide range of health benefits especially antimicrobial, anticancer, antihypertensive, antioxidant and anti – ageing properties so the research on bioactivity of secondary metabolites from Artocarpus genus can provide benefits in the search for new drugs of the natural material compound, as well as provide a scientific explanation of the use of these plants in traditional medicine [11-14].

Candida Microorganisms

Among the several hundred species of microorganisms in the oral cavity, yeasts, especially members of the genus Candida are normal commensal of the oral microbial flora and get established there during or soon after birth. These yeasts are commensal in healthy humans and may cause systemic infection in immuno – compromised situations due to their great adaptability to different host niches [15]. They are considered to be major human fungal pathogens that cause both mucosal and deep tissue infections. The prevalence of oral candidiasis in various count rise has been reported to range from 20 – 75 % [16]. The ability of Candida species to form drug resistant biofilms is an important factor in their contribution to human disease. Therefore, the incidence of fungal infections has increased significantly, contributing to morbidity and mortality [17,18].

Biofilms are structured microbial communities that are tightly attached to a surface and embedded within a matrix of extracellular polymers [17]. Biofilms are known to form on surfaces of catheters and prosthetic heart valves. In the oral cavity, yeast biofilms may form on acrylic dentures and dental implants. Fungal biofilms and their role in infection and drug resistance have received increasing amounts of interest in the past year. Yeasts are opportunistic pathogens and cause disease in hosts who are compromised by underlying local or systemic diseases. Oral candidiasis is a sign of impaired local or systemic defence mechanisms. As mentioned above candida organisms form biofilms on bioprosthetic surfaces and during the biofilm maturation they become highly resistant to antifungal drugs. The most commonly used antifungal drugs are azoles (fluconazole, itraconazole, and ketoconazole) and polyenes (amphotericin B) [17]. Some Candida species have intrinsic resistance and some develop resistance to azoles. Oral candidiasis is often found in the elderly, patients wearing dentures and HIV and AIDS patients. It is usually caused by Candida albicans (C. albicans), Candida tropicalis (C. tropicalis) and Candida glabrata (C. glabrata). All the different candida species cause the same kind of mucositis but there are significant differences in their invasiveness and antifungal susceptibility [17,18]. Present study has been aimed to evaluate the antifungal activity of Artocarpus heterophyllus (Jackfruit) latex and Cocos nucifera (Coconut) husk

Volume 2, Issue 2; March-April 2019; Page No. 152-164

extracts against C. albicans, C. tropicalis and C. glabrata and also to evaluate which solvent is most successful in extracting unknown antifungal compounds from the plant extracts.

Rationale – Selection of Plant, Pathogen and Solvent

The latex of Artocarpus heterophyllus and husk of Cocos nucifera were chosen based on the following facts: a) Both the plants are widely distributed tree in southern India [2], b) Though reports of antimicrobial activity are vastly available, only very few studies are available related to antifungal activity from the husk and latex, c) Easily available and economical, d) Folklore medicine claims that Cocos nucifera husk is used in treatment of diabetes and pneumonia [2]. Other species of Artocarpus had already proved to exhibit antimicrobial activity, e) Other parts such as roots, bark, oil from endocarp, leaf and seeds from both the plants have already been proved to exhibit antifungal activity [19-21] and f) In vivo studies done in rats had shown no toxic effects when administered orally [22]. The candida species were chosen for the following reasons: a) They are opportunistic microorganisms and are responsible for mucosal and systemic mycoses and one of the most pervasive pathogenic fungi. b) Especially they infect immuno-compromised hosts, which is a leading problem around the world. c) Due to increased drug resistance seen associated with Candida species against antifungal drugs. The choice of ethyl alcohol as a solvent is due to its solvency for a wide range of chemicals, its low antifungal activity and its low toxicity to mammals. Other available polar solvents namely water, methanol have been used in previous studies. The choice of di-ethyl ether as a solvent is due to its nonpolar nature and to isolate the non polar reactants from the crude plant extract. Ethers act as good organic solvents.

MATERIALS AND METHODS

The present in vitro study was conducted to evaluate the antifungal efficacy of the crude solvent extracts of Artocarpus heterophyllus latex and Cocos nucifera husk against three test organisms namely C. albicans, C. tropicalis and C. glabrata. The solvent extraction and antimicrobial bioassay of the selected plants was done in the Central Research Laboratory, Meenakshi Ammal Dental College & Hospital, Maduravoyal, Chennai.

Collection of Plants (Jack Fruit Latex and Coconut Husk)

Jack fruit and coconut trees are available plenty in Chennai city. The small branches of the jack fruit tree along with its leaves and leaflets of coconut tree along with husk were freshly cut and taken to the Department of Botany, Madras University, Chennai for taxonomical identification based on the botanical characters of the selected plants. After species identification, the husk of coconut was ground to coarse powder in an electronic mixer and the ground coarse powder was transferred to separate sterile bottles with stoppers. Latex of jack fruit was collected freshly in sterile bottles with stoppers by cutting the fruit with help of sterile knife. Each bottle was labelled with the name of the herb and was stored in a wooden cupboard. The standard fungal strains were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh by the Department of Microbiology, Meenakshi Ammal Dental College & Hospital, Maduravoyal, Chennai.

Preparation of Crude Extract

Using the organic solvents acetone, diethyl ether and ethyl alcohol crude extract preparation of two plants were carried out. About 25 g of the powdered husk (w/v) and jack fruit latex (v/v) were dissolved in 75 ml of the three solvents in glass bottles separately and were labelled appropriately. Acetone and ethyl alcohol were used for husk extract whereas diethyl ether and ethyl alcohol were used for latex extract preparations. Using the sterile glass rod, all the four constituents were mixed well and kept in orbital shaking incubator for 48 hours. All the extracts were filtered with the help of Whatman filter Paper (No.1) which was placed in a glass funnel and were collected in the conical flask. Following this, the extracts were transferred into preweighed petri plates which were marked and labelled. The extracts were left for evaporation for about 2 days. After complete evaporation, the plates were weighed again for detecting the amount of crude extract obtained from each solvent. Fully evaporated and solvent free crude extracts were then reconstituted in dimethyl sulfoxide, which is an inert organic solvent and prepared in a concentration of 100 mg/ml. These reconstituted extracts were then transferred to the sterile labelled glass vials and were stored in refrigerator (4° C) until use. Based on the solvents

Volume 2, Issue 2; March-April 2019; Page No. 152-164

used all the vials were pre labelled as follows: Husk acetone, husk ethyl alcohol, latex diethyl ether and latex ethyl alcohol.

Antibacterial Assay and Agar Well Diffusion Method

The cultures were added to Sabouraud's dextrose broth. The culture broth was incubated at 37° C for 2 hours. After incubation, the broths were used for the bioassay. The antifungal activity of the isolates on different extracts were tested using the agar well diffusion method. The cultures were standardized by McFarland standardization. A lawn culture was made onto Sabouraud's dextrose agar with sterile cotton swabs. Wells were punched onto the agar surface using agar puncture and the wells were loaded with four solvent extracts. Positive control (fluconazole) and negative control (dimethyl sulfoxide) were added in separate wells. 50 µl of the reconstituted extracts with a concentration of 100 mg/ml were loaded into each respective well using a micropipette. The plates were then incubated at 27º C for 24 hours. The extracts showing the zone of inhibition for each test organism was observed and was measured using a standard antibiotic zone measuring scale and recorded in millimetres. The bioassay was repeated for eleven times and the mean zone of inhibition was calculated.

Determination of Minimum Inhibitory (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) of solvent extracts of Cocos nucifera husk and Artocarpus heterophyllus latex were determined according to the micro broth dilution technique. It was performed in 96 – well microtiter plates for determining the MIC. Standardized suspensions of the test organisms were inoculated into a series of 96 – well micro titer plate, including one growth and one sterility control. The 50 µl/ml of broth containing plant extracts in increasing concentrations viz 6.25,12.5,25,50,100 mg/ml were pipetted to the corresponding well to which the preparations of the test organisms added and incubated at 37° C for 48 hours. After overnight incubation these tubes were observed for turbidity. The microtiter plate showing the minimum turbidity was noted for MIC. The test tube with the lowest dilution with no detectable growth/turbidity by visual inspection was considered the MIC. The minimum fungicidal concentration (MFC) values were determined by removing a loop full of fungal suspension from the MIC tubes that did not show any growth and sub -cultured. The plates were incubated at 37° C for 48 hours. After incubation, the concentration at which no visible growth was seen was recorded as the MFC.

RESULTS

The antifungal efficacy of Artocarpus heterophyllus latex and Cocos nucifera husk prepared in solvent extracts like ethyl alcohol, acetone and diethyl ether were evaluated against three candida organisms C. albicans, C. tropicalis and C. glabrata. The experiment was repeated for eleven times by Agar Well Diffusion method. The results were interpreted by measuring and recording the zone of inhibition for each test organism in millimetres. Quantitative variables obtained in the present study were assessed using Shapiro – Wilk Test. The zone of inhibition values for the positive control were parametric whereas for different solvent extracts found to be non parametric. The mean values of all the experiments were calculated and the data was statistically analyzed by one-way ANOVA. The intergroup comparison of different inhibition zones of different extracts were compared using Kruskal Wallis test. All the statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 16.0 [IBM Corporation, Chicago, IL, USA]. p value < 0.05 is considered to be statistically significant in the present study.

The MIC and MFC values obtained for Artocarpus heterophyllus latex and Cocos nucifera husk extracts against individual organisms are depicted in Table 1 – 3. The zone of inhibition measured for individual organisms against different solvent extracts of jack fruit latex and coconut husk are depicted in Table 4 – 7. The mean values of inhibitory zones obtained for individual organisms against each solvent extracts of jack fruit latex and coconut husk are represented individually in Table 5 and 7. Fluconazole was used as the standard antifungal agent for positive control and dimethyl sulfoxide was used as negative control.

For both the extracts, the values of MIC and MFC against C. albicans were observed between the range of 25 – 12.5 mg/ml and 50 – 25 mg/ml. Among the group, husk acetone extract was found to exhibit the least MIC and MFC values (Table 1 – C) whereas relatively higher MIC and MFC values are noticed in

Volume 2, Issue 2; March-April 2019; Page No. 152-164

latex diethyl ether, latex ethyl alcohol and husk ethyl alcohol extracts (Table 1 – A, B and D). These values signify that husk acetone extract was proved to have better fungi static and fungicidal effect against Candida albicans at much lower concentrations when compared with other solvent extracts.

As for the Candida tropicalis, MIC and MFC values were observed between the range of 25 – 12.5 mg/ml and 50 – 25 mg/ml respectively. While the acetone and ethyl alcohol husk extracts possessed the least MIC and MFC values (Table 2 – C, D), extracts of latex diethyl ether and ethyl alcohol possessed the maximum MIC as well as MFC values (Table 2 – A, B).

Similar to the results obtained in C. albicans, these values also suggest that the husk acetone extract exhibits better fungi static and fungicidal effect against C. tropicalis at much lower concentrations when compared with other solvent extracts. In case of C. glabrata, as seen in C. tropicalis the MIC and MFC values were observed between the range of 25 – 12.5 mg/ml and 50 – 25 mg/ml respectively. The least MIC and MFC values were noticed in both acetone and ethyl alcohol husk extracts (Table 3 – C, D). Similarly, maximum MIC and MFC values were found in diethyl ether and ethyl alcohol latex extracts (Table 3 – A, B).

These values also suggest that both the solvent extracts of husk (Table 3 – C, D) possessed better fungi static and fungicidal effect against C. glabrata at much lower concentrations when compared with other solvent extracts of latex. Addition to this, the results also shows that the observations found in C. glabrata differs from the results obtained for C. albicans and C. tropicalis, where among the different solvent extract groups (i.e. latex A, B and husk C, D) only husk acetone exhibited better fungi static and fungicidal effects (Table 1 – C, 2 – C). The zone of inhibition obtained for acetone and ethyl alcohol extracts of Cocos nucifera are represented in table 4. While ethyl alcohol and acetone husk extracts exhibited minimum zone of inhibition of 0 mm against C. tropicalis, maximum zone of inhibition of 17 mm was observed in husk acetone extract tested against C. glabrata (Table 4).

Zone of inhibition obtained from both the extracts exhibited the values from a minimum of 0 mm to a maximum of 14 mm (Table 4). Fluconazole, a standard antifungal agent was used as the positive control whereas dimethyl sulfoxide was used as negative control. The mean values of zone of inhibition observed for the individual species against each solvent extracts of Cocos nucifera husk are represented in table 5.

In Table 5, the mean zone of inhibition was found between 3.54 – 12.27 mm. The results showed that ethyl alcohol husk extract exhibited both minimum and maximum zone of inhibition. While the least mean value of 3.54 mm was observed against C. tropicalis, highest mean value of 12.27 mm was observed in C. albicans (Table 5). The zone of inhibition obtained for different solvent extracts of jack fruit latex against individual organisms are depicted in Table 6. Fluconazole was used as the standard antifungal agent for positive control and dimethyl sulfoxide was used as negative control.

The activity results showed that zone of inhibition was observed between 0 mm – 15 mm with ethyl alcohol and diethyl ether extracts showing minimum zone of inhibition of 0 mm against C. albicans and C. tropicalis. Maximum zone of inhibition of 15 mm was observed in ethyl alcohol latex extract against C. glabrata. The mean values of zone of inhibition observed for the individual species against each solvent extracts of Artocarpus heterophyllus latex are represented in table 7.

The mean zone of inhibition was observed between 1.09 – 9.6 mm. While diethyl ether latex extract exhibited minimum zone of inhibition of 1.09 mm against C. tropicalis, ethyl alcohol latex extract showed maximum zone of inhibition of 9.36 mm against C. glabrata (Table 7). From the results, it can be concluded that coconut husk acetone extract possessed stronger and almost equal inhibitory effect on C. albicans and C. glabrata with comparatively lesser inhibitory action on C. tropicalis (Table 5). As observed in acetone extract, ethyl alcohol extract of coconut husk also exhibited a similar pattern where it has exhibited maximum inhibitory effect on C. albicans followed by C. glabrata with comparatively lesser inhibitory action on C. tropicalis (Table 5). In case of Artocarpus heterophyllus, latex diethyl ether extracts exhibited substantial inhibitory effect on C. glabrata followed by C. albicans. Latex diethyl ether extracts proved to have a less inhibitory action on C. tropicalis (Table 7). The results represented in table 7

Volume 2, Issue 2; March-April 2019; Page No. 152-164

also showed that as observed in diethyl ether extract, latex ethyl alcohol too possessed maximum inhibitory effect on C. glabrata followed by C. albicans with comparatively less inhibitory action on C. tropicalis (Table 7). The Kruskal Wallis test was employed for carrying out the intergroup comparison of inhibition zones of different extracts (Table 8).

a. Kruskal Wallis Test, b. Grouping Variable: Groups

*p value of > 0.05, is considered as insignificant

*p value of < 0.05 is considered as significant

*p value of 0.00, is considered as highly significant

The results showed that a statistical significance of ($p < 0.05$) was observed among the four solvent extracts latex diethyl ether, latex ethyl alcohol, husk acetone and husk ethyl alcohol of Group A, Group B, Group C and Group D respectively against all the test organisms namely C. albicans, C. tropicalis and C. glabrata.

DISCUSSION

The key findings resulted from the agar well diffusion method proves that solvent extracts of husks of Cocos nucifera and latex of Artocarpus nucifera possessed reliable antifungal activity against all the three candida species (Table 1 – 8). They were more effective in inhibiting the growth of C. albicans and C. glabrata with less effective against C. tropicalis. Barring the ethyl alcohol husk extract tested against C. albicans (Table 1 – Group D), the average MIC and MFC values obtained for the solvent extracts tested against other candida species were found to be 12.5 and 25 mg/ml respectively (Table 1 – Group C, Table 2 – Group C, D and Table 3 – Group C, D).

Antifungal Evaluation – Cocos nucifera

Among the two solvent extracts, husk acetone extract has exhibited a minimum zone of inhibition of 0 mm against C. tropicalis and maximum zone of inhibition of 17 mm against C. glabrata (Table 4). Against C. albicans, both the extracts showed a significant antifungal activity with the least and substantial zone of inhibition were observed between the ranges of 8 mm to 15 mm for acetone and 10 mm to 14 mm for ethyl alcohol husk extracts (Table 4). The mean values of zone of inhibition exhibited by two solvent extracts against all the candida species were calculated (Table 5). From the results it has been clearly found that husk extracts of acetone and ethyl alcohol exhibited maximum activity towards C. albicans and C. glabrata followed by C. tropicalis. Both the extracts possessed significant inhibitory effect with mean vales of 11.63 (Table 5) and 9.09 (Table 7) on C. glabrata. Interestingly, against C. albicans husk extracts of acetone and ethyl alcohol exhibited almost equal inhibitory activity with the mean values of 11.9 and 12.2 (Table 5). As for the C. tropicalis, mean values of 3.72 and 3.54 (Table 5) were observed in husk extracts of acetone and ethyl alcohol respectively. The above mentioned statements interpret that coconut husk ethyl alcohol (mean value – 12.27) is more effective in inhibiting the growth of Candida albicans species in comparison with other two groups. This observation has been consistent with previous study done by M. Jose et al [23] who concluded that the husk fibre extracts inhibited the growth of C. albicans exhibiting a mean inhibitory zone of 6 mm. In contrast, based on their studies Rafaela et al [22] and Esquenazi et al [23] concluded that the growth of C. albicans were not inhibited by the husk fibre extracts. In order to find a better solvent, comparison of solvents has been done in the present study that could be optimally useful in isolating the antifungal components from crude plant extracts. Among the solvents used, acetone solvent extract showed the highest mean values with significant inhibitory zone against test organisms (Table 8). The crude extract of coconut husk prepared in acetone has proved to exhibit reliable and substantial antifungal activity. Generally, solvents like ethyl alcohol and acetone are considered to be best solvents in isolating phenolic components from plant extracts due to their polar nature [21] and this could be the reason for acetone solvent extract exhibiting a good antifungal activity in the present study.

Antifungal Evaluation – Artocarpus heterophyllus

The findings obtained from the agar well diffusion assay convey that the solvent extracts of latex are consistent with previous study done by Madhavi et al [25] and J. Siritapetawee et al [26]. On interpreting the MIC and MFC values it was observed that the solvent extracts of latex Artocarpus heterophyllus

Volume 2, Issue 2; March-April 2019; Page No. 152-164

were found to exhibit antifungal activity at a relatively higher concentration of 25 mg/ml – MIC and 50 mg/ml – MFC on all the three candida species (Table 1, 2 and 3 – A, B). When tested against C. albicans, latex in diethyl ether had shown the least zone of inhibition of 0 mm whereas ethyl alcohol latex has shown highest zone of inhibition of 14 mm (Table 6). In case of inhibition against C. tropicalis, while zone of inhibition with a least value of 0 mm was observed in both the solvent extracts, highest zone of inhibition of 7 mm was found in ethyl alcohol husk extract (Table 6). For C. glabrata, diethyl ether latex possessed the least zone of inhibition of 6 mm whereas ethyl alcohol latex found exhibiting maximum zone of inhibition of 15 mm (Table 6). The mean values of zone of inhibition exhibited by two solvent extracts against all the candida species were calculated (Table 7). The activity results showed that latex extracts of diethyl ether and ethyl alcohol exhibited maximum activity towards C. glabrata followed by C. albicans and C. tropicalis. Both the extracts possessed maximum and almost equal inhibitory effect possessing mean vales of 9.09 and 9.36 (Table 7) on C. glabrata. Similarly, against C. albicans diethyl ether extract showed a mean value of 6.909 whereas ethyl alcohol extract possessed a mean value of 8.18 (Table 7). As for the C. tropicalis, mean value of 1.09 and 2.0 (Table 7) was observed in latex extracts of diethyl ether and ethyl alcohol respectively.

In the present study, comparison of solvents has been done in order to find a better solvent that could be optimally useful in isolating the antifungal components from crude plant extracts. The solvents used in present study are diethyl ether and ethyl alcohol. Among the solvents used, latex extracts of ethyl alcohol found to exhibit a higher antifungal activity compared to that of diethyl ether. Due to their polar nature, ethyl alcohol has been considered to be one of the best solvents in isolating phenolic components from plant extracts [27]. Further alcohol provides a particularly effective way of maximizing the bioavailability of the antifungal component extracted from the plant [21]. This reason can be attributed for the alcohol extract to exhibit reliable and significant antifungal activity in the present study. Meanwhile diethyl ether used in present study showed inhibitory zone with least mean values due to its non polar nature. As a result, the phenolic compounds would not have been fully isolated resulting in less antifungal activity when compared to alcohol extract. Different mechanisms were proposed to explain antifungal activity of polyphenols. They are dependent on crucial extracellular microbial enzymes inhibition, growth inhibition by substrate deprivation or acting on direct metabolism through oxidative phosphorylation inhibition. The phytochemical assessment done by various researchers on latex of jack fruit revealed that latex is rich in phenolics, flavonoids and tannins [26,28-29]. The flavonoids which are successfully isolated from latex consist of the varied frameworks like chalcone, flavanone, flavan -3-ol, simple flavone, prenylflavone [29]. The antifungal properties of latex of jack fruit may be attributed by prenylflavones, namely cycloheterophyllin and artonins A and B. Prenylflavones are known to function as antifungal compounds through a number of mechanisms including radical scavenging by H - donation, prevention of chain initiation by donating electrons or by binding of transition metal ion catalysts [29]. Purified protein obtained from latex of Artocarpus plant exhibited protease activity by digesting gelatin and casein substrate and this may also contribute to antibacterial and antifungal activities of latex [29]. There might be a possibility that latex may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in ion leakage from the cell. Higher diffusion also occurs due to hydrophilic nature of fungal cell wall that results in an increase in antifungal properties of active components.

CONCLUSION

The purpose of this study is to evaluate the antifungal activity of husk of Cocos nucifera and latex of Artocarpus heterophyllus using different solvents like ethyl alcohol, acetone and diethyl ether against three microorganisms, namely C. albicans, C. tropicalis and C. glabrata. Agar well diffusion method was used to assess the antifungal activity and following which evaluation of mean values obtained from the zone of inhibition against test organisms were carried out. Results of this study showed that both the latex and husk extracts possessed higher antifungal activity against C. albicans and C. glabrata whereas found to be less effective against C. tropicalis. Acetone extract of Cocos nucifera husk had inhibited the growth of C. glabrata and C. tropicalis to a greater extent when

Volume 2, Issue 2; March-April 2019; Page No. 152-164

compared to other solvent extracts. Ethyl alcohol extract of Artocarpus heterophyllus had inhibited the growth of C. albicans to a greater extent when compared to other solvent extracts. From this study it has been concluded that solvent extracts of Cocos nucifera husk and Artocarpus heterophyllus latex were found to exhibit antifungal property particularly against candida species like C. albicans, C. tropicalis and C. glabrata. The major contribution of the antifungal activity of coconut husk extracts may be due to presence of polyphenols and tannins, as they are the major phytochemical constituents isolated during chemical analyses of husk in previous studies [22-23,30]. Major polyphenols like catechin, epicatechin, procynadin 1&2, Gallic acid and ellagic acid were isolated and reported previously from the fibre extract of coconut husk [22]. In particular, Gallic acid and ellagic acid were proved to exhibit antibacterial action which was further confirmed from the activity results obtained from the solvent extracts of coconut husk in the present study. However, the antifungal potency of husk extract of Cocos nucifera and latex extract of Artocarpus heterophyllus vary with the type of solvents and their activity with other solvents. Therefore, efficient separation methods for identification of active compounds must be evaluated in future research studies. Considering the crude nature and low toxicities of the solvent extracts used in this study, our results allow us to conclude that the crude extract from latex of Artocarpus heterophyllus exhibited significant antifungal activity and can be used in place of resistant antifungal drugs. The present study is time-saving, economical, effective, non-toxic and the samples are easily available. A typical research and developmental work need to be carried out for their better therapeutic and commercial utilization.

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Volume 2, Issue 2; March-April 2019; Page No. 152-164

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Volume 2, Issue 2; March-April 2019; Page No. 152-164

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Volume 2, Issue 2; March-April 2019; Page No. 152-164

Table 1: MIC and MFC values of latex of Artocarpus heterophyllus and Cocos nucifera husk extracts against C. albicans

I	Solvent	MIC	MFC
A	Latex diethyl ether	25 mg/ml	50 mg/ml
B	Latex ethyl alcohol	25 mg/ml	50 mg/ml
C	Husk acetone	12.5 mg/ml	25 mg/ml
D	Husk ethyl alcohol	25 mg/ml	50 mg/ml

Table 2: MIC and MFC values of latex of Artocarpus heterophyllus and Cocos nucifera husk extracts against C. tropicalis

I	Solvent	MIC	MFC
A	Latex diethyl ether	25 mg/ml	50 mg/ml
B	Latex ethyl alcohol	25 mg/ml	50 mg/ml
C	Husk acetone	12.5 mg/ml	25 mg/ml
D	Husk ethyl alcohol	12.5 mg/ml	25 mg/ml

Table 3: MIC and MFC values of latex of Artocarpus heterophyllus and Cocos nucifera husk extracts against C. glabrata

I	Solvent	MIC	MFC
A	Latex diethyl ether	25 mg/ml	50 mg/ml
B	Latex ethyl alcohol	25 mg/ml	50 mg/ml
C	Husk acetone	12.5 mg/ml	25 mg/ml
D	Husk ethyl alcohol	12.5 mg/ml	25 mg/ml

Table 4: Zone of Inhibition Exhibited by Acetone and Ethyl Alcohol Extracts of Cocos nucifera

	Zone of Inhibition in mm			
Test Organisms	Cocos nucifera		Positive Control	Negative Control
	Acetone	Ethyl alcohol		
C. albicans	12, 14, 10	14,14,12	25,28,25	NA
	15,11,8	12,14,13	25,25,25	NA
	9,14,14	11,10,12	28,28,25	NA

Volume 2, Issue 2; March-April 2019; Page No. 152-164

	14,10	13,10	30,29,30	NA
C. tropicalis	8,9,5,5 5,5,4,0 0,0,0	8,8,8 5,2,4 2,0,0 2,0	32,30,30 25,26,26 25,25,25 26,25	NA NA NA NA
C. glabrata	10,10,9 12,9,17 13,10,12 12,14	9,10,6 10,6,14 14,7,11 6,7	30,31,30 24,24,22 24,25,25 25,24	NA NA NA NA

NA-No activity

Table 5: Mean Values of Zone of Inhibition (mm) Exhibited by Acetone and Ethyl Alcohol Extracts of Cocos nucifera

Test Organisms	Cocos nucifera		Positive Control	Negative Control
	Acetone	Ethyl alcohol		
C. albicans	11.9	12.27	27.0	NA
C. tropicalis	3.72	3.54	26.0	NA
C. glabrata	11.63	9.09	26.0	NA

NA-No activity

Table 6: Zone of Inhibition Exhibited by Ethyl Alcohol and Diethyl Ether Extracts of Artocarpus heterophyllus

	Zone of Inhibition in mm			
Test Organisms	Jack Fruit Latex		Positive Control	Negative Control
	Diethyl ether	Ethyl alcohol		
C. albicans	10, 10, 13 13,5,8 9,8,0 0,0	11,12,14 12,9,8 9,6,2,5	25,28,25 25,25,25 28,28,25 30,29,30	NA NA NA NA
C. tropicalis	5,2,0 0,0,3 2,0,0 0,0	7,5,0 2,0,3,3 0,0,2,0	32,30,30 25,26,26 25,25,25 26,25	NA NA NA NA

Volume 2, Issue 2; March-April 2019; Page No. 152-164

C. glabrata	6,6,6	5,5,5	25,24,24	NA
	17,7,9	15,13	25,25,24	NA
	10,1,11	15,9,10	24,22,30	NA
	6,9	11,8,10	31,30	NA

NA-No activity

Table 7: Mean Values of Zone of Inhibition (mm) Exhibited by Ethyl Alcohol and Diethyl Ether Extracts of Artocarpus heterophyllus

Test Organisms	Jack Fruit Latex		Positive Control	Negative Control
	Diethyl ether	Ethyl alcohol		
C. albicans	6.9	8.18	27.00	NA
C. tropicalis	1.09	2.0	26.54	NA
C. glabrata	9.09	9.36	26.09	NA

NA-No activity

Table 8: Comparison of mean values of inhibition against the test organisms among the groups - Test Statistics[a,b]

	Group A (Latex Diethyl ether)	Group B (Latex Ethyl alcohol)	Group C (Husk Acetone)	Group D (Husk Ethyl Alcohol)
Chi- Square	15.438	16.376	20.669	20.419
df Asymp	2	2	2	2
Sig	0.000	0.000	0.000	0.000

Volume 2, Issue 2; March-April 2019; Page No. 152-164