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# Antimicrobial activities of polyphenol extract of arecanut against pathogenic bacteria

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The screening of plant extracts for antimicrobial activity has been of great interest in the search for new drugs suited for effective treatment of several diseases caused by microbes. Many plant extracts have antimicrobial principles such as tannins, essential oils and other aromatic compounds (Kumar and Singh, 1984). Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great therapeutic values.

*Areca catechu* L. is an important palm cultivated commercially in India and several south-eastern countries. Arecanut is reported to have pharmacological properties which may be attributed to its biochemical components such as polyphenols, alkaloids, polysaccharides, fat and proteins. Arecanut extract possess potential anti-oxidative activity (Kim *et al.*, 1997) and inhibition of free radicals and reactive oxygen species (Ohsugi, 1999). Arecanut extract was shown to have *in vitro* inhibitory effect on $H_2O_2$ induced RBC hemolysis and intestinal alpha–glucosidase enzymes like maltase and sucrase, followed by *in vivo* suppressive effect on post-prandial elevation in blood glucose in maltose tolerance test in rats (Senthil Amudhan and Hazeena Begum, 2005; 2008).

Main polyphenol subgroups in arecanut are proanthocyanidins, catechin, and epicatechin. Proanthocyanidins are high molecular weight flavonoid polymers (condensed tanins) while polyphenols are secondary metabolites widely seen in plants and its content varies between species, cultivars, maturity, season and region (Nonaka *et al*., 1981). Polyphenols are classified according to their structure as phenolic acid derivatives, flavonoids, and tannins. Polyphenols exhibit antibacterial activities with distinct characteristics in their reactivity with protein related polyamide polymers (Haslam, 1996). Arecoline is a major betel nut alkaloid (Arjungi 1976) of high pharmaceutical significance. Hydrolysable tannins present in *Areca* which include tannic acid were shown to possess antibacterial activity in several salivary and oral microorganisms (de Miranda, *et al.*, 1996). Extracts from *Areca* was reported to possess antimicrobial effects on *Helicobacter pylori* (Wank and Huang, 2005) and antibacterial activity on *Staphylococcus aureus*, *Salmonella* sp., *Neisseria* sp., *Yersinia enterocolitica*, and *Listeria monocytogenes* (Yang and Chou, 1997). However, studies on antimicrobial activity of arecanut biochemicals have not been carried out. The present study was undertaken to evaluate the antimicrobial activity of polyphenol and arecoline fractions in arecanut.

Arecanut (500 g) was ground into dry powder to which100 ml of 80% aqueous methanol was added, and the suspension stirred gently. Tubes were sonicated twice for 15 min and kept at room temperature for 24 h. The extract was centrifuged for 10 min and supernatant collected and filtered through Whatman filter paper, air dried and polyphenol content was estimated by using Folin–Ciocalteu reagent. (Swein and Hills, 1959). The extract was used for screening the antimicrobial activity.

Areca powder (10 g) was transferred to a separating funnel containing 20 ml distilled water and 1 ml of dilute sulfuric acid ($H_2SO_4$:$H_2O$ = 1:9). The solution was extracted with 25 ml portions of chloroform three times. Ammonia solution (1 ml)

was added and re-extracted with chloroform four times. Alkaline pH of the solution was maintained by adding ammonia solution, if required. The chloroform layer was drained into a conical flask using a separating funnel and the extraction was repeated till all the alkaloids were extracted into the chloroform. After evaporating the chloroform from the extract, a portion of it was used for quantification of arecoline, by dissolving in excess of 0.02 N $H_2SO_4$ and titrated against 0.02 N NaOH using methyl red as indicator (Nambudiri, 1968). The arecoline extract was filtered though Whatman filter paper which was used for screening antibacterial activity.

Human pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Shigella* and *Vibrio cholerae* were used for testing antibacterial activity. Microorganisms were obtained from the culture collections of Department of Microbiology, Kasturba Medical College, Manipal. Antibacterial activity was determined by the disc diffusion method (Bauer *et al,* 1966). A sterilized 6 mm diameter antibacterial susceptibility blank disc was loaded with 200 µL (4 mg) each of arecoline extract, (0.46%) synthetic arecoline HBr (0.46%), polyphenol (0.76 mg/mL), and the discs were dried in open sterile petri dish in a laminar air flow hood. The bacterium for testing was transferred onto a 9 cm diameter petri-dish containing nutrient-agar using a sterile cotton swab and spread over the whole surface of the medium as a thin film. The inhibition of bacterial growth was evaluated by measuring the diameter of the transparent inhibition zone around each disc. Control disc and standard antibiotic disc were loaded with the same solvent and dried using the same method as for the treated disc. All the experiments were repeated twice, including two controls with plain dimethyl sulfoxide (DMSO) and sterile distilled water all the time.

The total phenolic values were 164 mg/g dry weight and condensed tannins were 62.61 mg/g dry weight. Mature arecanut had high arecoline content (0.362 mg/g dry weight). The values represent mean of six replicates. It was found that mature nut contained high content of phenolic compounds. It is speculated that plants containing condensed tannins evolved over a time as a defense mechanism, against pathogenic microorganisms, insects and grazing animals (Swain, 1979). Four phenolic classes identified in arecanut include, phenolic acids, proanthocyanins, flavonols and flavan-3-ols, which consist of monomers and the polymer classes of proanthocyanidins. The polyphenols, mostly flavonols, comprise about 10 per cent (+) catechin, 2.5 per cent epicatechin, 12 per cent (+) leucocyanidin, the remaining portion being complex flavonoids in varying degrees of polymerization (Govindarajan and Mathew, 1963).

Antibacterial activities of arecanut fractions against human pathogens are shown in Table 1. The polyphenol and arecoline extracts were screened for antibacterial activity against six different human pathogenic bacterial species. The extracts of arecanut polyphenol inhibited the growth of *E.coli, S. aureus, Pseudomonas, V. cholerae* and *S. typhi* strains, while the extracts of arecoline had no effect on the growth of the above bacterial strains. However, synthetic arecoline, HBr showed moderate antimicrobial

**Table 1. Antibacterial properties of arecanut fractions against human pathogens**

| Microorganism | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 1% DMSO (control) | Arecoline (0.46%) in DMSO from arecanut | Synthetic Arecoline HBr | Water (control) | Polyphenol (7.6%) in water | Antibiotic Gentamycin |
| *E. coli* | * | * | 6 | * | 13 | 15 |
| *S. aureus* | * | * | 7 | * | 7 | 23 |
| *P. aeruginosa* | * | * | * | * | 10 | 15 |
| *V. cholerae* | * | * | 8 | * | 9 | 14 |
| *S. typhi* | * | * | 7 | * | 9 | 22 |
| *Shigella* spp. | * | * | * | * | * | 20 |

*No inhibition

activity. The inhibition zone of arecanut polyphenol extract against *E. coli* or *Pseudomonas* strains showed higher inhibition which is comparable with antibiotic gentamycin while other bacterial strains showed moderate inhibitiory zone. No zones of inhibition were found with regard to dimethyl sulfoxide (DMSO) and water control indicating no antimicrobial activity of these solvents used in this study. The present study is in agreement with other similar studies conducted earlier showing antibacterial activity of arecanut ethanol extracts (Yang and Chou, 1997). Another study showed that a variety of human and veterinary microorganism isolates, both gram positive and gram negative were inhibited by aqueous extract of arecanut (Reena *et al*., 2010).

In this study, polyphenol extract of arecanut showed significant bactericidal or bacteriostatic activities. Catechol and pyrogallol, both hydroxlyated phenols, were shown to be toxic to microorganisms. Catechol has three -OH groups, the site and number of these thought to be related to the relative toxicity to microorganisms (Gessiman, 1963). In addition, some authors have found that highly oxidized phenol is showing more inhibitory effect. The mechanism thought to be responsible for phenolic toxicity to microorganisms include, enzyme inhibition by oxidized compounds, possibly through reaction with sulfhydryl groups or non-specific interaction with proteins (Manson and Waserman, 1987). The inhibition of microorganisms by phenolic compounds may also be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes (Scalbert, 1991). Among the phenolic compounds, proanthocyanidins are vulnerable to polymerization in air through oxidization. Therefore, an important factor governing the toxicity of proanthocyanidins is their polymer status. Oxidized condensation of phenols may result in the toxification of microorganisms. On the other hand, polymerization can result in the detoxification of phenols (Scalbert, 1991; Field and Lettinga, 1992). Hence, it is suggested that polyphenols present in the arecanut extract might be responsible for the antimicrobial activities. Further studies are needed to understand the exact mechanism in the antimicrobial property of arecanut polyphenols which may have immense potential in the pharmaceutical industries.

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*Central Plantation Crops Research Institute,*
*Regional station, Vittal, Karnataka - 574 243*

M. Senthil Amudhan

*Corresponding Author: senjp@yahoo.com*