Coconut - powerful to fight against cancer

Nevin K.G
Assistant Professor, DBT-MSUB, School of Biosciences, Mahatma Gandhi University, PD Hills P.O, Kottayam-686560. Email. nevinkg@gmail.com, Tel:9645631807

Research on the effects of dietary polyphenols on human health has developed considerably in the past several years. Various studies strongly support a significant role for polyphenols in the prevention of cardiovascular diseases and several types of cancer. Although the antioxidant properties of polyphenols have been widely explored, it has now become clear that the mechanisms of action of plant derived polyphenols go beyond the modulation of oxidative stress.

Apart from having antioxidant properties, polyphenols have several other specific biological actions that are poorly understood. Several studies in different laboratories aim to establish evidence for the direct link between polyphenol consumption and health. Such studies also help us to identify the individual polyphenolic component from the existing complex mixture of polyphenols which provide the greatest protection in the context of preventive nutrition. If these goals are to be attained, it is essential to determine the nature and distribution of these compounds in our diet. Such knowledge will allow evaluating the polyphenol intake and enabling epidemiologic analysis that will in turn provide an understanding of the relation between the intake of these substances and the risk of development of diseases.

Coconut and its products, especially oil is a part of human diet for several centuries. Although the fatty acids present in coconut oil was always a subject of controversy, a very few scientific data is available on the nature and properties of minor components present in coconut kernel. Previous studies have showed that virgin coconut oil (VCO) contains significant amount of polyphenols which have the capacity to prevent the oxidation of low density lipoprotein (LDL) in *in vitro* conditions and provides significant radical scavenging activity.

Since the minor components from the whole kernel are not subjected to detailed studies, a study was undertaken which designed to isolate, characterise and evaluate the radical scavenging and anti cancer effect of polyphenols rich extract (*PPr*) from the kernel of coconut.

Isolation of polyhenol rich extract: Polyphenol rich extract from west coast tall variety coconut kernel (*PPr*) was prepared with 100% methanol using soxhlet apparatus by the procedure shown in (Figure 1);

Qualitative phytochemical analysis: *PPr* was evaluated for the presence of biologically active components viz. phenolic compounds (Lead acetate test), flavonoids (Alkaline reagent test), tannins (Ferric chloride test), steroids, triterpenoids (Salkowski's test), saponins (Froth test), cardiac glycosides (Keller Killiani test) and alkaloids (Wagner's test) using standard procedures. Total polyphenols and flavonoids were estimated colorimetrically. The results are shown in figure2.

Figure 1. Extraction of PPr from coconut kernel

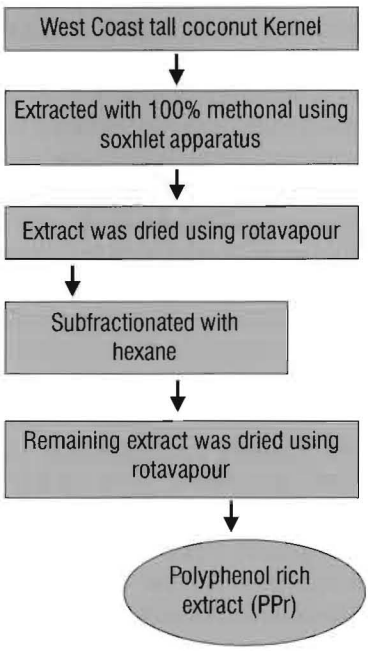
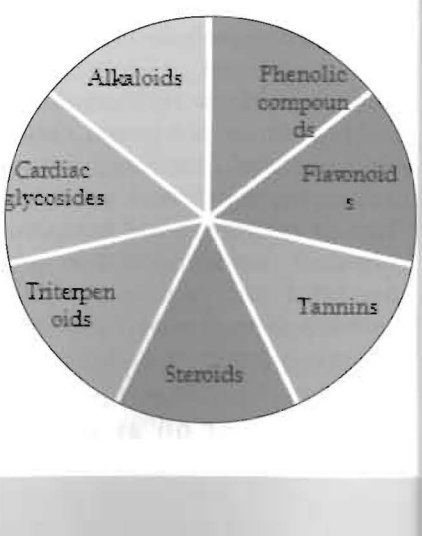
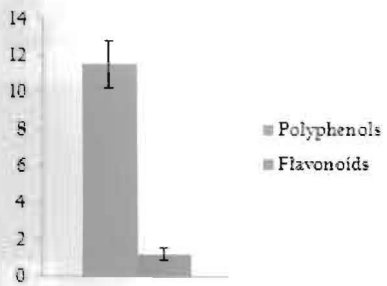


Figure 2. Qualitative phytochemical composition, total polyphenols and flavonoid content of PPr





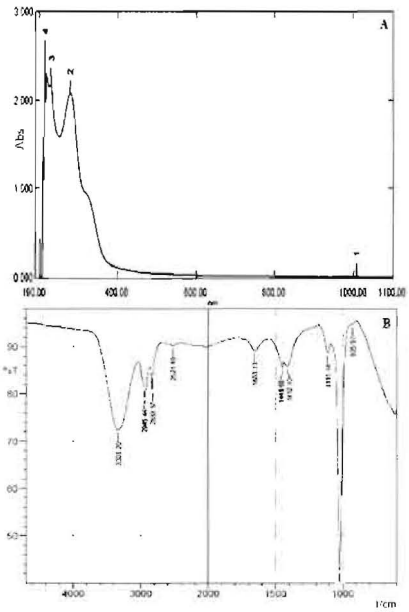
Values are mean ± SD of three independent estimations; Polyhenols are expressed as Gallic acid Equivalace (GAE) mg/100 g; Flavonoids are expressed as Quercetin Equivalance (QE)mg/100 g

Preliminary phytochemical analysis showed that *PPr* contains phenolic compounds, flavonoids, tannins, steroids, titerpeniods, cardiac glycosides and alkaloids. Colorimetric estimation showed very high amount of polyphenolic compounds compared to flavonoids. The other components may be present in trace amounts compared to polyphenols.

UV visible and FTIR analysis of *PPr*: UV visible spectrum of *PPr* in methanol was recorded using Shimadzu UV visible spectrophotometer (Model UV-2600, ISR -2600 plus). FTIR analysis of *PPr* in methanol was recorded using Shimadzu IR prestige 21 with ATR. The results are shown in figure 3.

UV visible spectrum of *PPr* showed several peaks between 209nm and 579 nm. The absorbance at 215, 230 and 279nm may correspond to the presence of polyphenolic compounds and flavonoids. FTIR analysis of *PPr* showed characteristic absorption bands at 3334 & 3300 cm for hydroxyl (-OH) groups corresponding to polyphenolic compounds, 2945 & 2833 cm (for C-H stretching), 1448 cm (for C-H bending), and at 1641 cm for C=C group.

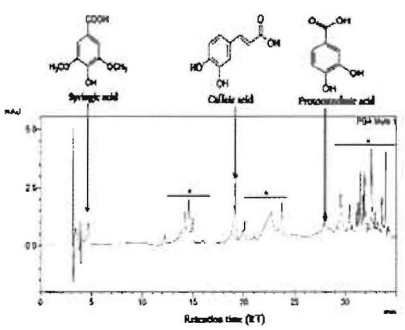
Figure 3. UV visible and FTIR analysis of PPr



A-UV-Vis Spectrum, B: FTIR spectrum

HPLC Analysis: The phenolic composition of *PPr* was determined by HPLC Shimadzu UFLC DGU-20As systems coupled to quaternary rapid separation pump (LC- 20AD), ultimately SIL-20A HT autosampler and rapid separation PDA detector (SPD-M20A), as described by Uddin et al,2014 with slight modifications. Phenolic profiling of *PPr* was done using a C18 (250 × 4.6 mm) column. The mobile phase consisted of water (Solvent A) at pH 3 adjusted with acetic acid, acetonitrile (Solvent B) and methanol (Solvent C). The system was run with the following gradient elution program: 0 min, 5%A/95%B (10 min), 10%A/80%B/10%C (20min), 20%A/60%B/20%C (30 min), 100%A. There was a 5 min post run at initial conditions for equilibration of the column. Pure standards were used to confirm the presence of compounds. The results are given in figure 4.

Figure 4. HPLC analysis of PPr

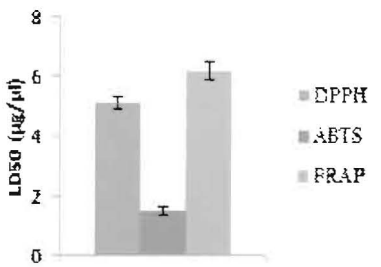


**Indicated un identified peaks*

Phenolic profiling using HPLC showed several unidentified peaks, while the presence of syringic acid (RT- 4.703), caffeic acid (RT-19.131) and protocatechuic acid (RT-27.954) was confirmed using pure standards.

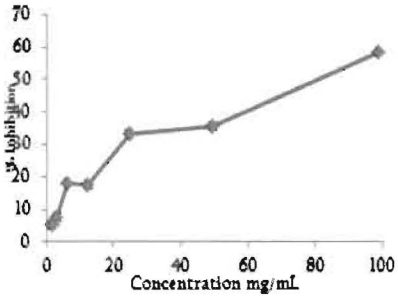
In vitro radical scavenging assays: The free radical scavenging activity of *PPr* was tested using DPPH, ABTS and FRAP method. The results are shown in figure5.. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH- solution in the presence of a hydrogen donating antioxidant. ABTS $^{\cdot+}$ scavenging is considered as an electron (e) transfer reaction. Ferric ion reducing antioxidant power (FRAP) is an antioxidant capacity assay that uses Trolox as a standard. This method is usually used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols.

Figure 5. DPPH, ABTS and FRAP activity of PPr



All data are means of three (n = 3) independent measurements ± SD

Figure 6. In vitro anti lipid peroxidation effect of PPr



Values expressed as ± SD of three separate experiments

Antioxidant activity depends on the number and position of the hydroxyl groups of the aromatic ring binding site and the type of substituent. The phenolic compounds are good reducing agent since the principle antioxidant activity of this compound sort is due to their redox properties. PPr can interact with the DPPH radical and convert it into non-radical (DPPH-H) yellow colored diphenyl-picrylhydrazine by donating hydroxyl group. The LD_{50} value was found to be 5.1μg/μl. Previous studies have showed that caffeic acid, protocatechuic acid and syringic acid have significant DPPH radical scavenging activity.

In vitro lipid peroxidation: A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the preventive effect of *PPr on* lipid peroxide formed, using liver homogenate as lipid rich medium. The results are showed in figure 6.

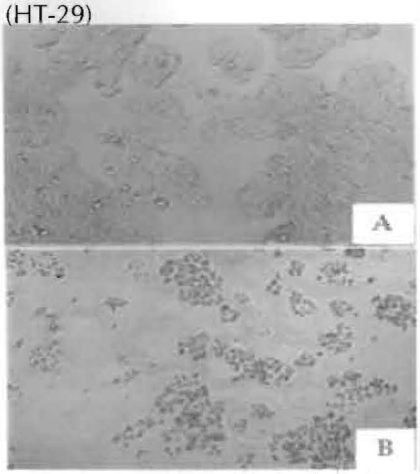
The results showed that *PPr* have significant effect in preventing the peroxidation of hepatic lipids indicated by low TBARS levels.

Cytotoxicity Assay: Anti cancer effect of *PPr* was evaluated in vitro using human colon cancer cells HT-29 procuired form nation Center for Cell sciences (NCCS), Pune.

In vitro cytotoxicity of *PPr* (5mg/ml; 72 hrs incubation) against HT 29 cell showed that *PPr* is capable of killing the cells as evidenced from the sulphorhodamine B assay as well as using microscopical observation (Shown in figure 6). PPr treated cells showed more non adhered floating cells; indication of cell death, compared to adherent control cells.

PPr isolated from coconut kernel contains phenolic acids *viz* syringic acid, caffec acid and prorocatechuic acid. This extract also showed significant radical scavenging effect as evidenced from DPH, ABTS and FRAP assay. The present findings showed that *PPr* with its biologically active polyphenolic components can be useful for human nutritional purposes. A further study is required to elucidate the ability of *PPr* against oxidative stress in *in vivo* models.

Figure 7. Cytotoxixicty of PPr against human colon cancer cells (HT-29)



Cytotoxic activity of polyphenolic fractions from coconut kernel (5mg/ml). A: Control, B: PPr treated

Antimicrobial Properties of Coconut Oil on *Candida* Species in Ibadan, Nigeria

The emergence of antimicrobial resistance, coupled with the availability of fewer antifungal agents with fungicidal actions, prompted a study to characterize *Candida* species in the environment and determine the effectiveness of virgin coconut oil as an antifungal agent on these species. In 2004, 52 recent isolates of *Candida* species were obtained from clinical specimens sent to the Medical Microbiology Laboratory, University College Hospital, Ibadan, Nigeria. Their susceptibilities to virgin coconut oil and fluconazole were studied by using the agar-well diffusion technique. *Candida albicans* was the most common isolate from clinical specimens. *C. albicans* had the highest susceptibility to coconut oil (100%), with a minimum inhibitory concentration (MIC) of 25% (1:4 dilution), while fluconazole had 100% susceptibility at an MIC of 64 ìg/mL (1:2 dilution). *C. krusei* showed the highest resistance to coconut oil with an MIC of 100% (undiluted), while fluconazole had an MIC of >128 ìg/mL. It is noteworthy that coconut oil was active against species of *Candida* at 100% concentration compared to fluconazole. Coconut oil can be used in the treatment of fungal infections in view of emerging drug-resistant *Candida* species.

Study conducted by:

Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Ogun State, Nigeria