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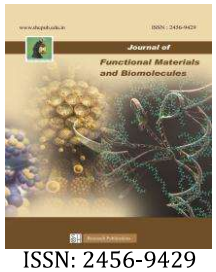

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Phytochemical screening and antioxidant property of Cocos nucifera sheath

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Abstract

Unpleasant side effects associated with prolonged use of current antioxidant, anti-inflammatory drugs have necessitated a need for new drugs with limited side effects. Plants rich in antioxidants have been suggested as potential sources of anti-inflammatory compounds. Thus, the anti-inflammatory and antioxidant activities of extracts of *Cocos nucifera* sheath with widespread use in folkloric medicine in India were evaluated in this study. The phytochemical composition of aqueous and ethanol extracts of C. *nucifera* sheath were determined using standard methods. Antioxidant potential was screened using the DPPH assays. The anti-inflammatory activities were evaluated using an *in vivo* assay. Aqueous sheath extract established the existence of carbohydrates, quinines, terphenoids and steroids, whereas ethanol extracts contains carbohydrates, tannins, saponins, alkaloids, quinines, terphenoids and steroids. Dose dependently the plant extracts shows the potential free radical scavenging effect against DPPH radical. Ethanol and aqueous extracts scavenges the free radicals effectively from concentration ranges of 20-100 µg/ml, which shows 20-55% of DPPH radical inhibition. Aqueous and ethanol extracts of C. *nucifera* sheath shows significant anti-inflammatory activity at dose dependent manner. The activity is compared with diclophenac sodium, a standard anti-inflammatory drug. The ethanol extract at the concentration of 100-500µg shows 10-53% inhibition of protein denaturation. Hence the present study suggests that *Cocos nucifera* sheath may be used as a potential source to treat oxidative stress and tissue injury related problems.

Keywords: antioxidant; anti-inflammatory; Cocos nucifera sheath; protein denaturation, oxidative stress.

1. Introduction

Free radicals surpass the level of antioxidants in living organisms which leads to develop oxidative stress. Free radicals are the molecules possess unpaired electron in their configuration. This imbalanced consequence leads to develop several diseases due to damage in DNA, protein and lipids through the chemical reaction called oxidation [Wu et al., 2013]. Mild to moderate level these oxidants may exert beneficial effects to the biological systems; however at high concentration it can be harmful, responsible for onset and progession of diabetes, cancer, atherosclerosis, metabolic defects and cardiovascular disease [Taniyama and Griendling, 2003]. Radicals like singlet oxygen (1O2), Superoxide radicals (O2•−), hydroxyl radicals (•OH) and hydrogen peroxide (H2O2) are the most common reactive oxygen species (ROS) in living organisms which are generated during their metabolic process as a by-product [Sato et al., 2013]. Phosphorylation of proteins, apoptosis of cells, immunity, cell differentiation and activation of factors regulating transcription process are depends on enough production of ROS in living system and should reside inside of the cell at low level.

Mitochondria serves as a major cell organelle for the production of ROS, through some pathological and physiological events such as cellular respiration during metabolism of arachidonic acid; can leads to generate superoxide radicals by LOX (lipoxygenases) and COX (cyclooxygenases) in inflammatory and endothelial cells [Al-Gubory et al., 2012]. Though these organelles possess natural free radical scavenging capacity, cannot able to clear the entire amount of mitochondrial ROS [Hansen et al., 2006]. Living

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cells deploy defense mechanism to protect injury from ROS is termed as antioxidant system mainly based on enzymatic machinery like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic machinery like Vitamin C and Vitamin E [Deponte, 2013]. These antioxidants can act as an electron donor, donates electron to free radicals thereby produce stable molecule with less reactive nature.

Active inflammatory cells at inflammatory site enhance the synthesis of enzymes such as protease, collagenase, elastase, lipases, acid hydrolase, phosphatase and highly reactive free radicals like superoxide, hydroxyl radical, hydrogen peroxide, and mediator chemicals such as complement component, nitric oxide, chemokines, cytokines, eicosanoids; which collectively cause oxidative stress and tissue damage. The process involved in inflammation can activates phagocytosis through macrophages and neutrophils leads to generate large quantity of ROS and RNS (reactive nitrogen species) like superoxide, nitric oxide, hydrogen peroxide peroxynitrite and hydroxyl radicals to neutralize the effect of invading agents [Fialkow et al., 2007]. Uncontrolled formation of ROS and RNS during inflammatory condition and diffuse out of these reactive species can develop oxidative stress and tissue injury in the affected area. Proinflammatory cytokines responds to nonphagocytic cells also responsible for the production highly reactive oxygen and nitrogen species [Wu et al., 2013; Li et al., 2015]. Hence, it clearly evidenced that the inflammatory process can persuade oxidative damage to the cells, the oxidative stress can also induce inflammation through activation of multiple pathways.

Several anti-inflammatories as well as antioxidant drugs were formulated ant used for the ailment of oxidative stress, tissue inflammation and its related diseases, however none is found to potent, because they can cause undesirable side effects to the biological organisms. Hence researchers mainly focused on plant-based material to treat oxidative stress and tissue injury since they possess less toxic nature when compared to commercially available drugs. One such plant is Cocos nucifera (L.) Arecaceae family plant has used traditional medicine but lack of scientific security. Hence in our study we planned to explore the phytochemical composition, antioxidant and anti-inflammatory activities of aqueous and ethanol extract of Cocos nucifera Sheath.

Figure 1: *Cocos nucifera* sheath

2. MATERIAL AND METHODS

2.1 Chemicals

Ethanol, DPPH, Ascorbic Acid, Sodium Chloride (NaCl), Potassium Chloride (KCl), Disodium Hydrogen Phosphate (Na2HPO4), Potassium Dihydrogen Phosphate (KH2PO4), Hydrochloric Acid (HCl), Hen's Egg, Diclofenac Sodium were purchased from Mumbai.

2.2 Collection and preparation of Cocos nucifera sheath

Sheath of cocos nucifera were collected from trees near Pudupet village, Tirupattur district, Tamil Nadu, India. Sheath were collected and allowed to shade dry for about one week. After that dried sheath are grind well to obtain a powder. 10mg of sheath powder was mixed with 100 ml of distilled water and ethanol, separately to prepare aqueous and ethanol extracts, respectively. Then the resultant mixture was kept under rotary incubator for up to 24 hours. After completion of the incubation the mixture was filtered and used for further analysis.

2.3 Qualitative phytochemical screening

The phytochemical analysis of aqueous and ethanolic extract of cocos nucifera sheath was performed using a standard method. This screening was done to give a gen-

eral overview of the several groups of chemicals that were present in the algal extract. The extracts are utilized in additional research [Harbone 1998 and Kokate 2001].

2.4 Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay by brand Williams et al. (1995)

The free radical scavenging potential of aqueous and ethanolic sheath extract was determined using DPPH [Brand Williams et al., 1995]. The DPPH solution (0.006% w/v) was prepared in methanol. Different concentrations of aqueous and ethanolic sheath extract (50, 100, 150, 200 and 250 µg/ml) was prepared. Algal extracts were mixed with 300µl of DPPH solution in dark room. Ascorbic acid, which is a strong antioxidizing agent is taken as standard, prepared in different concentrations using distilled water (50, 100, 150, 200 and 250 µg/ml). Different concentration of ascorbic acid was taken with 300 µl of DPPH solution in dark room. The prepared solution of ascorbic acid and algal extracts were incubated for 30 minutes and then absorbance was measured using UV Spectrophotometer at 517 nm. Methanol and DPPH serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula;

DPPH radical scavenging activity (%) = (Control OD-Sample OD) / Control OD x 100

2.5Anti-inflammatory activity by egg albumin denaturation assay

Inhibition of egg albumin denaturation was determined using the method prescribed by chandra, et al 2012.

To prepare phosphate buffer saline (pH 6.4) 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na2HPO4), and 0.24 g of potassium dihydrogen phosphate (KH2PO4) were dissolved in 800 ml of distilled water. The pH was adjusted to 6.4 using 1N hydrochloric acid (HCl) and made the volume to 1000 ml with distilled water. Control solution (5 ml): 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (pH 6.4), and 2 ml of distilled water. Standard solution (5 ml): 0.2 ml of egg albumin, 2.8 ml of phosphate buffer, and 2 ml of various concentrations of the standard drug diclofenac sodium (50-250 µg/ml). Test solution (5 ml): 0.2 ml of egg albumin, 2.8 ml of phosphate buffer, and 2 ml of various concentrations of test samples (50-250 µg/ml).

Procedure

2.8 ml of phosphate buffer (pH 6.4) and 0.2ml of egg albumin were incubated with various concentrations (50,100,150,200 and 250 µg/ml) of test samples and standard drug diclofenac sodium (50,100,150,200 and 250 µg/ml) then the samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula.

Percentage inhibition = (Abs control − Abs sample)/ Abs control × 100

3. RESULTS AND DISCUSSION

The qualitative phytochemical analysis revealed the presence of numerous biologically important secondary metabolites both in aqueous and ethanol extract of Cocos nucifera sheath was depicted in table 1.

Table 1: Qualitative Phytochemical Analysis of C. *nucifera* sheath extracts

S. No	Phytochemicals	Aqueous extract	Ethanol extract
1	Carbohydrates	+	+
2	Tannins	-	+
3	Saponins	-	+
4	Alkaloids	-	+
5	Flavonoids	-	-
6	Glycosides	-	-
7	Quinones	+	+

8	Phenols	-	-
9	Terpenoids	+	+
10	Steroids	+	+

+ indicates presence - indicates absence

Aqueous sheath extract established the existence of carbohydrates, quinines, terphenoids and steroids, whereas ethanol extracts contains carbohydrates, tannins, saponins, alkaloids, quinines, terphenoids and steroids. Non-nutrient biologically vital secondary metabolites are derived mainly from ecology; it exerts numerous beneficial as well as pharmacological properties to the health of humans. Plant secondary metabolites are organic compounds not involved directly in the regular development, growth and organism's reproduction. Phytochemicals plays a major role in the plant's adaptation to the environment but it characterizes chief foundation of pharmaceuticals (Nasir Mahmood *et al*., 2019). The qualitative analysis of secondary metabolites in aqueous and ethanol extracts of C. *nucifera* proved that the sheath extracts possess various important phytochemicals, which readily accounts for the traditional medicinal uses of this C. *nucifera* sheaths. By nature alkaloids possess significant pharmacological effects to human well-being. Most of the artificial and semi-synthetic medicines are derived mainly from slight modifications from the structure of alkaloids. Heterocyclic nitrogen compound alkaloid mainly derived from tyrosine, tryptophan and lysine. Alkaloids exert wide range of pharmacological activities which includes antibiotics, anticancer and several degenerative diseases. By these vast pharmacological properties, alkaloids play a potent part in the design of pharmaceutical medications particularly for inflammatory disorders and cancer [Kaur and Arora, 2015]. As a secondary metabolite, alkaloids believed to perform a self-protective role against various pathogens and herbivores. Owing to their significant biological activity, nearly 12000 alkaloids are known to exploit as stimulants, poisons, pharmaceuticals and narcotics.

Sterols encompass numerous chief clusters of steroids categorized by having functional hydroxyl moiety at carbon-3, having β configuration and at the C-17 it has 8-10 or even more atoms of carbon in their side chains. Steroids most predominantly occurred in the kingdom of plant as well as animals. Sterols act as important membrane constituents by its structural role and play a key role in biosynthetic sequences which lead to the steroidal species. For the plant biosynthesis, sterols act as starting material. Sterols cannot formed by human system, so it consumed only through diet. Several reports suggested that sterols decrease the level of cholesterol in serum. The sterols derived from plants serves as chief source for insecticides, steroid compound, anticancer and antioxidant formulations. Several reports suggest that the phytosterol shaving potent anti-cancer of ovary, stomach, lungs and breast cancer and anti-inflammatory and antioxidant activity.

The large complex biomolecule having polyphenol nature possess enormous hydroxyl groups along with other suitable subgroups like carboxyl are termed as tannins. Carboxyl groups involves in the formation of strong complex with various macromolecules (Navarrete 2013). Tannins used in the ink making, tanning, dyeing and in medicine. Provisionally tannins are termed as, compound extracted from plant origin owing the property to convert skins of animals into leathers. By their chemical properties they were classified into hydrolysable and condensed tannins. Biologically most significant secondary metabolite saponins found enormously in plants, herbs, beans and vegetable (Francis *et al*., 2002; Haralampidis *et al*. 2002). Saponins contains wide variety of structure by sugar chain and aglycones, it possess various pharmacological and biological activities and act as an important active ingredient in ancient medicines. Terpenoids, synthesised by plant kingdom as a tiny molecular product has potent pharmacological properties such as antibacterial, anti-inflammatory, antiviral, antimalarial, anticancer and cholesterol synthesis inhibition.

In-vitro antioxidant activity of aqueous and ethanol extracts of C. *Nucifera* sheath were depicted in figure 2. Dose dependently the plant extracts shows the potential free radical scavenging effect against DPPH radical. Ethanol and aqueous extracts scavenges the free radicals effectively from concentration ranges of 20-100 µg/ml, which shows 20-55% of DPPH radical inhibition. The basis of this antiradical activity assay is the reduction of DPPH* (DPPH radicals) under dark environment in the solution of ethanol. At 517 nm, the odd electron arrangement in DPPH displays solid maximum absorption.

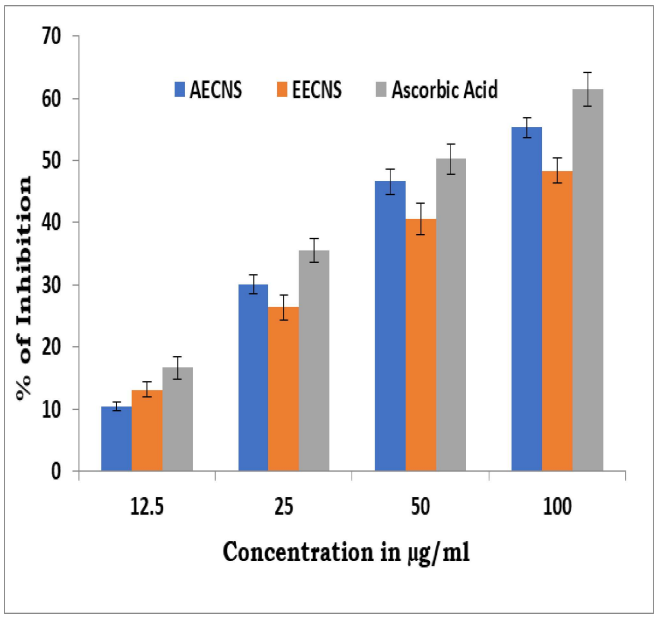

Figure 2: in-vitro antioxidant activity of C. *nucifera* sheath extracts

Hydrogen donor molecules such as antioxidants having free radical scavenging effect provides the electron to free radicals, thus makes free radicals odd electrons to become paired off. This electron pairing in the fee radicals leads to decreased absorption strength, resulting decolorization is stoichiometric with respect to free radical captured electrons number. The free radical scavenging activities of extracts depend on the capacity of antioxidant compounds to lose hydrogen and the structural conformation of these components. Ascorbic acid serves as standard antioxidant compound. Nitrogen centered stale free radial DPPH can adopt hydrogen or electron radical in order to become a constant diamagnetic molecule. DPPH radicals act with appropriate reducing agents, then depriving colour stoichometrically with the number of electrons depleted which is measured spectrophotometricallty at 517 nm (Karou *et al*., 2005). When compared to aqueous sheath extract, ethanol extracts possess significant DPPH scavenging activity. At the concentration of 100µg, ethanolic sheath extract scavenges more than 55% of the DPPH radicals.

The anti-inflammatory activity of the sheath extract is depicted in figure 3. Aqueous and ethanol extracts of *C. nucifera* sheath shows significant anti-inflammatory activity at dose dependent manner. The activity is compared with diclophenac sodium, a standard anti-inflammatory drug. The ethanol extract at the concentration of 100-500µg shows 10-53% inhibition of protein denaturation.

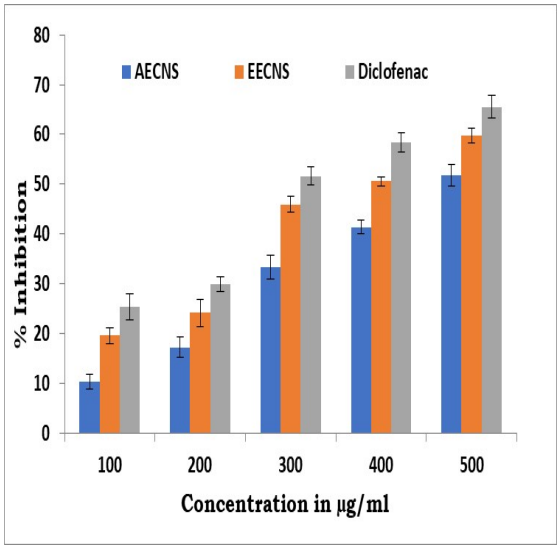

Figure 3: Anti-inflammatory activity of C. *nucifera* sheath extracts

In the development of some arthritic diseases, denaturation of the proteins found in the tissues may cause the auto-antigen production, which causes the tissue injury. Hence denaturation of tissue protein is thought to be a significant clinical marker for tissue inflammation and disease like arthritis. Protein denaturation preventing agents

could be potential candidate for the development of anti-inflammatory drugs. In view of this point, in the current study, the protein denaturation bioassay study was selected to assess the *in-vitro* anti-inflammatory activity of sheath extracts with a wide range of dose concentrations. The data obtained from the present findings shows that the sheath extracts exhibit dose dependent inhibition of protein denaturation. The increased absorbance in ethanol and aqueous extracts and the standard drug indicates the inhibition of protein denaturation (increase the protein stabilizing activity) with increased concentration.

4. CONCLUSION

The *Cocos nucifera* sheath extracts possess various secondary metabolites such as alkaloids, tannins, steroids, terpenoids, saponins, quinines and carbohydrates which were evidenced by their qualitative phytochemical analysis. The both aqueous and ethanol extracts of *Cocos nucifera* sheath showed a significant antioxidant activity compared to standard ascorbic acid by diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method. When compared to standard anti-inflammatory drug diclofenac, plant extracts displayed dose dependant inhibition of protein denaturation. The significant antioxidant and anti-inflammatory activity of *Cocos nucifera* sheath extracts mainly attributed by its various biological important secondary metabolites. Hence the present study suggests that *Cocos nucifera* sheath may be used as a potential source to treat oxidative stress and tissue injury related problems.

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