ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 6, pp. 17661-17665, June, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ISOLATION, CHARACTERIZATION AND PHARMACOLOGICAL EFFECT OF BIOACTIVE COMPOUND FROM TWO VARIETIES OF COCUS NUCIFERA L

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0806.0400

ARTICLE INFO

Article History:

Received 18th March, 2017
Received in revised form 10th April, 2017
Accepted 06th May, 2017
Published online 28th June, 2017

Key Words:

Antitumour, Topoisomerase Enzymes, Antiprotease

ABSTRACT

Medicinal plants represent a vast potential source for anticancer compounds. These compounds are extremely complex molecular structures, which would be difficult to synthesize (or conceptualize) in the laboratory. The antitumour activity of medicinal plant derived compounds may result via a number of mechanisms, including effects on cytoskeletal proteins which play a key role in cell division, inhibition of DNA topoisomerase enzymes, antiprotease or antioxidant activity, stimulation of the immune system, etc. (1). Plants can delay or even prevent cancer on-set. Plants can support the immune system, thus improving body resistance to the disease and its treatments. Plants can prevent and decrease side effects of conventional treatments. Plants can provide nutritional, as well as psychological support.

INTRODUCTION

Natural products are generally either of periodic origin or originate from microbes, plants, or animal sources Plants produce primary and secondary metabolites. Primary metabolites are those chemicals produced by plants that are directly involved in plant growth and development. They are essential for survival as glucose and chlorophyll. In contrast, secondary metabolites do not appear to be essential for survival. Plants are recognized for their ability to produce a wealth of secondary metabolites that are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections

Bioactive Compounds

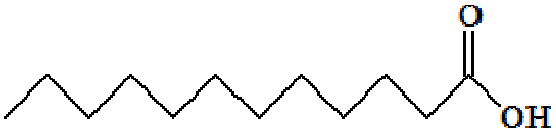
Secondary metabolites are produced by plants allegedly because they play certain biological and/or ecological roles towards combating other plants, animals, insects and man. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents for centuries to treat a variety of diseases or serve as the starting point in the development of modern medicines. The primary health benefits of coconut milk are due to the presence of lauric acid. There is very little lauric acid in the modern diet. Immune benefits: Lauric acid is transformed into monolaurin in the body. Monolaurin has also been shown to have some anti cancer effects.

Lauric acid

Pure coconut oil contains about 50 percent lauric acid, and is the most abundant natural source of lauric acid available. The lauric acid's anti-oxidant quality prevents the cancerous cells formation in the breast. Medium chain triglycerides featured in unadulterated coconut and coconut milk do not raise serum cholesterol nor contribute to heart disease.

Lauric acid is also known to the pharmaceutical industry for its excellent antimicrobial properties, and the monoglyceride derivative of lauric acid, monolaurin, is known to have even more potent antimicrobial properties against lipid-coated RNA and DNA viruses, numerous pathogenic gram-positive bacteria, and various pathogenic protozoa.

Structure of Lauric acid



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When lauric acid is present in the body, it is converted into monolaurin, a monoglyceride compound which exhibits antiviral, antimicrobial, antiprotozoal and antifungal properties. It acts by disrupting the lipid membranes in organisms like fungus, bacteria and viruses, thus destroying them.

Scientific Classification (Aliyar nagar 1)

Kingdom : Plantae
Subkingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Class : Liliopsida
Subclass : Arecidae
Order : Arecales
Family : Arecaceae
Genus : *Cocos*
Species : *Cocos nucifera L.*
Variety : Cocos nucifera L.Var.typica (Tall)

Tall : **Aliyar Nagar 1 or ALR (CN) 1**
Time take for bearing : 5 years
Average Yield : 126 nuts / palm / year
Copra content : 131 gram / nut
Oil content : 66.5 per cent

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Division : Magnoliophyta
Class : Liliopsida
Subclass : Arecidae
Order : Arecales
Family : Arecaceae
Genus : *Cocos*
Species : *Cocos nucifera L.*
Varity : Chandrasankara (COD x WCT)

Selection from: Chowghat Orange Dwarf x West Coast Tall
Time take for bearing: 3 to 4 years
Average Yield: 116 nuts / palm / year
Copra content: 215 gram / nut
Oil content: 68 per cent

Objectives

Cocos nucifera is used in the ayurvedic system of medicine. Several medicinal properties have been attributed for the plant and only few phytoconstituents have been reported and lauric acid being the major fatty acid. Traditional uses associated with as well as properties portrayed by non-indigenous species, suggested that the *in vitro* anti-oxidant, anticancer and antimicrobial activity of the 10 selected species indigenous to should be determined. The cytotoxicity of each of the extracts was investigated and the compounds responsible for the selected biological activities isolated and identified.

The aims of the present investigations are as follows

- Collection of plant-(Hybrid and Normal coconut fruit)
- Authentication of plant material
- Phytochemical Investigation of secondary metabolites
- Chromatographic studies using Column and TLC
- Fractionation
- Characterization of isolated compound (HPLC, GCMS)
- Nutrient analysis
- Protein, carbohydrate, lipid, Vitamin C, Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium and Zinc.

To evaluate the following pharmacological and microbiological activities

- In-vitro antioxidant activity carrying out of anti-oxidant assay. Antibacterial activity Antifungal activity Anti ulcer activity.
- Determination of cytotoxicity of each of the plant species using the MTT assay.

METHODOLOGY

The sample coconuts were collected from papanasam taulk from Thanjavur District, Tamil Nadu, India. The collected cocnuts were shade dried for 15 days and powdered.

Extraction of Plant Material

The 20g of the shade dried coconut powder material was crushed and extracted by cold percolation method using Aqueous and Ethanol sequentially at 24 hrs. The extract was filtered using Whatmann filter paper and concentrated. The extract was put in airtight container and stored in refrigerator which was subjected to following analysis.

Quantitative Analysis
TLC

Thin layer chromatography is one of the valuable and versatile methods for analysis of wide rang biomolecules. TLC is nothing but a modification of paper chromatography. Where the sheet of paper is replaced by thin layer of absorbent material. Therefore the separation in TLC is also due to the differential partition of solutes between the stationary and mobile phases.

Principle

The general principle involved in TLC is similar to that of column chromatography, i.e., adsorption chromatography. In the adsorption process the solute competes with the solvent for the surfaces sites of adsorbent. Depending on the distribution coefficients the compounds are distributed on the surface of the adsorbent of course, in TLC the partition effect in the separation is also not ruled out The adsorbent normally used contains a binding agents such as calcium Sulphate which facilitates the holding of the adsorbent to the glass plate.

Procedure

The stationary phase is prepared as slurry with water or buffer at 1: 2 and applied to a glass plate or an inert plastic or aluminum sheet, as thin uniform layer by means of a spreader such as glass rod or pipette or using a TLC applicator. (0.25 mm thickness for analytical separations and 2-5 mm thickness for preparative separations are prepared).

Plate Development

The chromatographic tank is filled with developing solvent to depth of ~1.5 cm and equilibrated for about 5 hrs. The thin layer plate is placed gently in the tank and allowed to stand for about 60 min. make sure the spots do not touch the solvent directly capillary action caused the solvent to ascend as in paper chromatography and the separation of compounds takes place. As the solvent front reaches about 1-2 cm from the top of the plate, the plate is removed, solvent front is marked with a pencil immediately and allowed to air dry placing the plate upside down.

RESULTS AND DISCUSSION

Phytochemical screening

The present study carried out on two varieties of *Cocosnucifera, L.*Aliyarnagar1 and Chandrasankara variety extracts with aqueous, the phytochemical compound screened by qualitative method. In the phytochemical analysis, seven bio active compounds namely Alkaloids, Carbohydrates, Saponins, Tannins, Steroidal Glycosides, Phenols and Terpenoids are present in the Aliyarnagar1 aqueous extract, but in case Chandrasankara variety five bio active compounds namely Saponins, Flavonoids, Coumarin, Anthocyanin and Flavones were detected.

Qualitative Analysis Aliyar nagar1 and Chandrasankara variety

S.No	Name of the Test	Phytochemical constituents	Aliyar nagar1	Chandrasankara variety
1	Mayer's test Dragondraff test Wagner Test	Alkaloids	+ + +	+ + +
2	Molish Test	Carbohydrates	+	-
3	H_2SO_4	Saponins	-	-
4	Lead Acetate	Tannins	+	+
5	Salkowaski	Steroidal Glycosides	+	+
6	Ammonia	Flavonoids	-	-
7	Phenol reagent	Phenols	+	+
8	Chloroform and H_2So_4	Terpenoid	+	+
9	10% Nacl	Coumarin	-	-
10	NaOH	Anthocyanin	-	-
11	Hcl	Flavones	-	-

Isolation of lauric acid by Chromatography technique

In this study lauric acid was isolated and purified by the column chromatography. The isolated lauric acid shows 0.95% of R_f value in TLC study. The isolated compound was elucidated by ^{1}HNMR, ^{13}CNMR, IR and MS as well as comparison of the analytical data.

The greater speed of TLC is due to the more compact nature of the adsorbent, while working with labile compounds. Finally, the sensitivity of TLC is such that separations on less than milligram amounts of material can be achieved if necessary.

Spectrum analysis of lauric acid

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in (Table.4). The FTIR spectrum profile was illustrated in the. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in extracts. During the research endeavor, lauric acid was isolated from *Cocusnucifera* by Chromatography method and identified by FTIR and NMR Spectra. IR and NMR (Nuclear Magnetic Resonance) spectral analysis of eluted bands of lauric acid was presented inFig-14. Infrared spectra of lauric revealed a broad OH group absorption at 3421.72 cm^{-1}, strong CH_2 absorptions at 2924.09 and 2854.65cm^{-1} and absorptions at 1720.50 cm^{-1} attributed to vibrations of methyl and methylene groups in the sterol molecule and the fatty acid chain and broad absorptions in the region of 1103- 964.41cm^{-1} characteristic of sugar. The spectra showed amide with NH and CO absorptions at 1639.49 or 1523.76 cm^{-1}.

The fatty acid compounds were identified in coconut milkby GC-MS analysis. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in. The prevailing compound 9, 12- Octadecadienoic acid (Z, Z)-(CAS) Lauric acid (1.60%) was mostly found in GCMS study.

Nutrient and Mineral Analysis

The present study depict the comparative nutrient analysis such as Protein, Carbohydrate and cholesterol , mineral and vitamins were analyzed in the Aliyarnagar1 and Chandrasankara variety products.

The present study estimated the minerals such as Iron, Calcium, Magnesium, and Phosphorous in compounds. Minerals are the electrical transmitters in our body system. The better mobilization of minerals like Calcium, Magnesium, and Phosphorous in the body is very useful for the problems like calcium depletion due to various reasons. Minerals make it best anti ageing supplement which can maintain youthful vigor and vitality forever.

IN-VITRO antioxidant activity by power reducing assay

The antioxidant property of the product was analyzed by Power reducing assay. All the organs, fluids in the reproductive system are made up of cell need protection. Antioxidants can provide protection from these free radicals which keeps the DNA intact thus results in healthy eggs for conception.

In-Vitro Anti-microbial activity of lauric acid

The antimicrobial property of the lauric acid was tested against ten bacterial species and five fungal species by using two different varieties namely, Aliyarnagar1and Chandrasankara variety the fortnightly data of present study were observed, recorded and tabulated. The antimicrobial property of lauric acid isolated from coconut milk was tested against ten bacterial species and five fungal species by disc diffusion method. The zone of inhibition was assessed and the plates were kept at room temperature for 24-48 hours. The microbial species namely, *Pseudomonas aeruginosa, Bacillus cereus, Aeromonashydrophila, Shigellaflexneri, V. choleraeElT, V. cholerae 0139, Salmonella typhii, V. cholerae classical, V. cholerae 01790* and *E. coli* (ETEC) and also some selected fungus species such as *Rhizoctoniasolani, Fusariumudum, Macrophominaphaseolina, Alternariaalternata* and

Sclerotiumrolfsii were used to evaluate the inhibitory activity of Lauric acid.

***In-Vitro* Anti-bacterial activity of Lauric acid**

S. No	Name of organisms	Inhibition values in mm	
		A	S
1	*Pseudomonas aeruginosa*	-	-
2	*Bacillus cereus*	7	11
3	*Aeromonashydrophilla*	-	-
4	*Shigellaflexneri*	-	-
5	*Vibrio choleraeElT*	-	-
6	*V. cholerae 0139*	-	-
7	*Salmonella typhi*	-	-
8	*V. cholerae classical*	7	-
9	*V. cholerae 01790*	7	7
10	*E. coli* (ETEC)	-	-

A-Antibiotic, S – Sample

The present investigation shows the maximum antibacterial activity was recorded for lauric acid against *Bacillus cereus* (11mm) whereas minimum inhibitory activity was recorded *V. cholerae 01790* (7mm) against lauric acid respectively. There is no inhibition for *Pseudomonas aeruginosa, Aeromonashydrophilla, Shigellaflexneri, Vibrio choleraeElT, V. cholerae 0139, Salmonella typhi, Salmonella typhi, V.cholerae classical* and *E. coli* (ETEC).

***In-Vitro* Anti fungal activity of lauric acid**

S. No	Name of organisms	Inhibition values in mm	
		A	S
11	*Rhizactoniasolani*	12	7
12	*Fusariumudum*	11	-
13	*Macrophominaphaseolina*	12	6
14	*Alterneriaalternata*	11	9
15	*Sclerotiumroysii*	12	-

A-Antibiotic, S – Sample

The maximum inhibitory activity of lauric acid was observed against *Alterneriaalternata* (9mm) and minimum activity was noted against *Macrophominaphaseolina* (6mm) whereas no inhibition zone present in against *Fusariumudum,* and *Sclerotiumrolfsii* respectively.

In the present investigation, the anti-cancer activity of lauric acid was screened from 250 to 500 µg/ml of concentrations with the dilution leads to 1:1 to 1:2 using the selected sample. The results unearthed the capacity of the anti cancer potential of the selected sample such as lauric acid at two different dilutions which further exhibits the withstanding capacity of the cells was minimum in the higher concentrations when compared to lower concentrations. Both the varieties at different dilutions reacts exactly the same when comes to cell viability. In 1:2 at 250(µg/ml) dilutions concentrations both the varieties the cell viability were found to be 18.22 and 19.63, Where as in the 1:1 at 500 (µg/ml) dilutions concentrations the cell viability were observed at 9.12, 14.22 respectively. In general, the cell proliferation was controlled more in normal Coconut milk then hybrid Coconut milk particularly in higher concentration.

SUMMARY AND CONCLUSION

Science has long acknowledged the value of healing substances found in nature, such as digitalis, aspirin, penicillin, insulin, steroids, etc. There has been a resurgence of interest, both scientifically and popularly, in the utilization of natural approaches.

Coconut milk in India and world belonged to the sub family species *Cocos nucifera* have highest medicinal uses. This traditional medicinal knowledge had been supplanted by a conventional medical system, which, despite relying on many of the therapeutic compounds derived from traditional or alternative medical paradigm. Biomedical and pharmacological studies of coconut tree inflorescence are still lagging behind. This present study suggests that a continued focused effort on ethno pharmacological studies in milk would be a prerequisite for future advances in drug development from coconut palms tree inflorescence.

In order to elucidate the intrinsic ability of the coconut milk , two different types has been allowed for all the investigations hence proved our native coconut milk nurtures well than the hybrid cocnut which further advocating the need to promote the native coconuts in the future and safe guarding the species against all odds.

Evaluation of nutrients has been done in both the sample 1 and sample 2 namely, Protein, Carbohydrate, Lipid, Iron, Phosphorous, Magnesium, Calcium and Vitamins. The mineral and nutrient levels are comparatively rich in sample 1 than sample 2.

The Antioxidant capacity of the samples has been estimated using Power reducing method shows the sample 1 has higher value when compared with sample 2. The anti-oxidant capacity of the samples interprets that the anti-ulcer activity of the test compounds were perhaps the results of the interplay between their cytoprotection and the antioxidant properties.

The susceptibility power of the desired samples itself could pave the way for the cell lines since the basic capacity of the compound against the microbial species could clearly depicts the strong withstanding power of the desired samples. It has been strongly advocated even after various trials using different methods. Whereas the difference in antibacterial activity, by disc diffusion method, between sample 1, sample 2 and control recorded drastically that is the sample 1, sample 2 recorded high inhibition rates when as to control. When comes to antifungal activity of all the respective formulations of extracts of coconut milk sample 1 and sample 2 were screened for the in vitro growth inhibitory activity against *Rhizoctonia solani, Fusarium udum, Macrophomina phaseolina, Alternaria alternata and Sclerotium rolfsii* by using agar disc diffusion method. In conclusion, the overall results found to be replica of the anti bacterial capacity that is sample 1, sample 2 played authoritative roles when compared to control in anti fungal activity as well. But in both the cases the inhibition rate was high in sample 1 than sample2.

Cancer cells usually display enhanced glucose uptake under hypoxic conditions, due to over expression of glycolytic enzymes and glucose transporters, as compared to healthy cells. Therefore, attaching a carbohydrate functionality to a metal-

arene moiety appears a promising strategy to exploit biochemical and metabolic functions used for transport and accumulation of sugars in living organisms.

The anti cancer properties of the samples using cell line delivers the very positive results by inhibiting the cell proliferation even in different dilutions cementing the very purpose of using novel drug delivery systems by incorporating the cocnut milk particularly lauric acid, thus making it as a potential option for management against breast cancer.

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How to cite this article:

Thangaraj N and Jayalakshmi C., Isolation, Characterization And Pharmacological Effect of Bioactive Compound from two Varieties of Cocus Nucifera L. *Int J Recent Sci Res.* 8(6), pp. 17661-17665.
DOI: http://dx.doi.org/10.24327/ijrsr.2017.0806.0400

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