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Evaluation of In Vitro Antioxidant and Pharmacological Activities of Carica papaya (Red Lady) Leaf Extracts with Physiological Relevance: Therapeutic Applications.

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ABSTRACT

Background: Medicinal plants surge from traditional use, particularly in developing countries, and their antioxidant potential as natural pharmaceutical-alternatives and health-enhancing diets. Papaya, is known for its edible fruits and medicinal properties, with its leaves, especially the Red Lady (RL) variety, being highly therapeutic.

Objective: This study compares the antioxidant activity of acetone and n-hexane extracts from Carica papaya leaves (RL).

Methods: This experimental study was conducted at the Department of Pharmacology and Therapeutics at Baqai Medical College/University of Karachi, utilizing in-vitro techniques, spanned duration of 6months. The protocol entails preparing pure samples at 0.5mM in DMSO and crude samples at 0.5mg/ml in DMSO. A 0.3mM DPPH solution in ethanol is prepared using DPPH. The test involves mixing 95µl of the 300µM DPPH solution with 5µl of the 500µM test solution and incubating the mixture for 30minutes at 37ºC. Absorbance is measured at 517nm using a Spectra-Max-340-multi-plate-reader. During the reaction, the solution changes color from violet to pale-yellow, indicating reduction.

Results: Acetone extract exhibited modest activity with 52.8%, 53.1% and 53.8% inhibition, but the n-hexane extract illustrate no activity, representing -8.5%, -9.0% and 9.2% -Inhibitions, while, Gallic Acid showed significant inhibition, reaching 95.0%, 95.3% and 95.6% at a concentration of 0.5mg/ml. Moreover, the acetone extract achieved from the Carica Papaya Leaves (RL) determined an IC50±SEM value of 476.4±0.51µg/mL, whereas gallic acid showed an IC50 value of 3.69µg/mL ($p < 0.001$).

Conclusion: These results propose that the acetone extract from the Carica Papaya Leaves (RL) might possess certain anti-oxidant activity, whereas it is less potent than the standard drug.

Keywords: *Carica papaya, Antioxidant, n-Hexane, Acetone, Gallic Acid, Physiology, Pharmacology*

INTRODUCTION

Our body depend on greatly on oxygen (O_2) to competently metabolize biomolecules and create energy (1). In contrast, the consumption of O_2 likewise produces reactive oxygen species (ROS), which, while essential for life, can be extremely toxic and harmful to cells. Antioxidants work an important part in neutralizing ROS and modifying oxidative-stress before it produces harm to tissues. The environment has produced various endogenous antioxidant systems in reactions to ROS production. While, it has developed into evident that these intrinsic protection systems are frequently insufficient to manage with ROS under particular circumstances. Consequently, there is developing importance in strengthening the body natural antioxidant defense mechanism by way of the combination of exogenous antioxidants. These exogenous resources consist of vitamins and synthetic agents, and their supplement is having faith in to help lessen the development of oxidative injury and associated ailments (2,3). Antioxidants, which reduce the effect ROS and secure cells from oxidative injury, are crucial for keep up cellular homeostasis and general wellbeing. Apprehensions approaching the protection and efficacy of synthetic antioxidants have built up curiosity in natural substitutes, predominantly medicinal plants (4).

Carica papaya, a fruit harvest refined in tropical and sub-tropical areas world-wide, be a member of the Caricaceae family. While, this fruit is very appreciated for its remarkable nutritious and medicinal properties (5), include anti-bacterial (6), anti-viral, anti-helminthic (7), anti-hemolytic (8), and immune enhancing (9) effects. In the beginning farmed in the lowlands of Central-America and southern-Mexico, it is currently cultivated significantly in various tropical and subtropical areas worldwide (10). *Carica Papaya* is a lush source of important vitamins, consist of A-B-C-K (11), with β-carotene (12), roughage or bulk (13), C6H12O6 (carbohydrates), and essential minerals for instance Na-K-Fe-Ca-Mg-Mn-(mangane), Co-(cobalt), S-(sulfur), P-(phosphorus), and Zn-(zinc)(14). On the other hand, the ripe-fruit is frequently utilized, the unripe-fruit fulfills as a nutritive vegetable (15). Additionally, the *Carica papaya leaves* remain consumed as vegetables and for preparing tea (16). Although, papaya leaves are plentiful in anti-oxidants, including phenolics and flavonoids, augmenting their nutritious role (17). In this research concentrated on the *Carica papaya (RL) variety*, a gynodioecious F1 hybrid plant well-known for its reddish orange fruit pulp and aromatic fragrance (18). This variety was preferred for its unique characteristics.

Carica Papaya leaves (RL) be full of above fifty-bio-active compounds, constructing them important for controlling different human ailments (19). Scientific researchers have recognized plentiful quantities of alkaloids, flavonoids, glycosides, saponins, phenolic components, amino acids, carbohydrates, lipids, enzymes, vitamins, and minerals in leaves of papaya (20). Although it is fruit often garners the most attention, the leaves of papaya are regularly over-looked and discarded. Research has exhibited that leaf extracts of papaya possess a range of valuable effects. This study aimed to assess the DPPH radical scavenging activity of acetone and n-hexane extracts derived from *C. papaya leaves*, with a particular focus on the *Red Lady variety*, and to investigate their potential protective effects against oxidative stress.

MATERIAL AND METHOD

Study Design: This experimental study was conducted at the Department of Pharmacology and Therapeutics at Baqai Medical College/University of Karachi, utilizing in-vitro techniques. The research spanned duration of 6months.

Plant Collection and Identification: Specimens of the Carica papaya 'Red Lady' variety (CPL. var. RL) were gathered from recent harvests at the University of Karachi and were subjected to authentication by an ethno-botanist. The plant was assigned an official identification code (G.H.#97627).

Extraction of Plant Material: The extraction method for the *Carica papaya 'Red Lady' variety (CPL var. RL)* was carried out at the International Center for Chemical and Biological Science, Karachi University, Pakistan. Initially, five-kg of green leaves of *CPL var. RL* were collected, washing with tap and distilled water, the *Carica papaya leaves* go through air drying, chopping, and blending processes. Acetone and n-hexane were consumed as solvents in a Soxhlet extractor, as well as 10gm and 82gm of dried sample developed for acetone and n-hexane extractions, correspondingly. The extraction method, which reach over 72hours, comprised cycling the solvent vapor by way of the sample, be a consequence by condensation and collection of the extract. Subsequently, the sample was condensed beneath reduced pressure at 60°C by means of a Buchi-Rotavapor R200. While extracts dried and saved in a refrigerator for prior examination. This specific process makes sure comprehensive extraction of constituents, preparing them for succeeding exploration (21).

2,2-diphenyl-1-picrylhydrazyl (DPPH) RADICAL SCAVENGING ASSAY: In this research conducted by DPPH radical scavenging process, recognized for its simplicity, quickness, and sensitivity in calculate approximately the antioxidant activity of a specific compound or plant extract. A dark-colored crystalline powder comprising of stable free-radical particles, works as the corner-stone of this method. Comprehensively used in laboratory research, primarily in anti-oxidant assays, DPPH establishes a deep-violet color in its radical condition when dispersed in ethanol, as well as strong absorbance detected at 517nm. Upon interaction through an anti-oxidant for instance Vitamin C, DPPH experiences reduction to its molecular form (DPPHH), subsequent in a noticeable alteration in color to pale-yellow supplemented by diminished absorbance. This variation in absorbance works as a metric for defining the test sample ability to scavenge radicals, giving perceptions into its anti-oxidant activities.

PROCEDURE

Pure-sample: 0.5mM in Dimethyl-sulfoxide (DMSO)

Crude-Sample: 0.5mg/ml in Dimethyl-sulfoxide (DMSO)

2,2-diphenyl-1-picrylhydrazyl (DPPH) Solution-(Wako Chemicals USA, Inc.): 0.3mM in Ethanol

A mixture comprising 5µL of 500µM test solution and 95µL of 300µM DPPH solution in ethanol is incubated for 30minutes at 37ºC to allow the reaction to progress. The absorbance of the consequential mixture is examined by means of a Spectra-Max-340-multiplate-reader at 517nm. During the reaction, solution color transitions from bluish-purple to pale-yellow as it undergoes reduction. The IC_{50} value represents the concentration at which a 50% reduction in the initial DPPH concentration occurs. To determine the IC_{50} values of various components, the Enzyme-Kinetics-analysis-program (EZ Fit, Perrella Scientific Inc., Amherst, MA, USA) have being employed (22,23). Gallic Acid served as the reference compound in these analyses.

Statistical Analysis: Data will be percentages and IC_{50}±Standard error of the mean (SEM), based on triplicate parallel measurements and analyzed One-Way ANOVA followed by Tukey's HSD post-hoc test.

RESULTS

In this study, we evaluated the antioxidant ability of the *Carica Papaya Leaves (RL)* by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging process, with Gallic Acid serving as the standard. DPPH, a stable free-radical with a purple-color, undergoes conversion to its non-radical form, DPPH-H, upon interaction with antioxidants. As a result, the purple color of DPPH decreases, turning into a light-yellow color. The extent of color change imitates the usefulness of the plant extracts in neutralizing free radicals.

The study assessed the DPPH free-radical scavenging ability of acetone and n-hexane extracts from the *Carica Papaya Leaves (RL)* at different concentrations with the DPPH method. Results showed that the acetone extract demonstrated modest activity with 52.8%, 53.1% and 53.8% inhibition, at the same time as the n-hexane extract exhibited no activity, showing -8.5%, -9.0% and 9.2% -Inhibitions. In comparison, the standard drug Gallic Acid demonstrated significant inhibition of 95.0%, 95.3% and 95.6% at a concentration of 0.5mg/ml, as illustrated in the table. Additionally, the acetone extract from the *CPL var. RL Leaves* exhibited an IC50±SEM value of 476.4±0.51µg/ml, while Gallic Acid showed an IC50 value of 3.69µg/ml. Tukey's post-hoc test showed that the antioxidant activity of gallic acid was significantly greater compared to acetone extract ($p < 0.001$) and n-hexane extract ($p < 0.001$). Moreover, the acetone extract was more active than the n-hexane extract ($p < 0.001$).

TABLE-I-Antioxidant Activity of Difference Conc. of Various Extracts from *CPL Var. RL Leave*

Sample	% Inhibition	% Inhibition	% Inhibition	IC_{50}±SEM	Mean Diff.	p-value
***Acetone ext.* 0.5mg/ml**	52.8	53.1	53.8	476.4±0.51 µg/ml	**Acet vs Std** 42.2	<0.001
***n-hexane ext.* 0.5mg/ml**	-8.5	-9.0	-9.2	-	**n-Hex vs Std** 104.3	
Gallic Acid (Hd) 0.5mg/ml	95.0	95.3	95.6	3.69µg/ml	**Acet vs n-Hex** 62.1	

DISCUSSION:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging process, have being employed to evaluate the in-vitro anti-oxidant properties of acetone and n-hexane extracts achieved from *CPL var. RL Leave* at various strengths, together with Gallic Acid, also called 3,4,5-trihydroxy-benzoic acid, is a poly-phenolic constituent frequently found in different plants, fruits and nuts(24,25). In addition to much poly-phenols, gallic acid is a low molecular-weight tri-phenolic constituent

by remaining anti-inflammatory and anti-oxidative activities(26).

The results from the table emphasize that the acetone extract determined insignificant activity, while the n-hexane extract enlightened no considerable ability for free radicals scavenging in relationship to the standard drug (Std.). These values underscore the variable levels of anti-oxidant activity detected between the samples and the Std. drug, proposing an ability divergence in their efficacy in free radicals scavenging. This investigation is imperative, principally in relation of the reported high strength of particular anti-oxidants discovered in acetone extracts from *CPL var. RL Leaves*. In 2020, reported by Prakit Chaithada et al., it revealed that the ethanol extract acquired from *RL leaves* established the most significant DPPH free-radical-scavenging property, with an IC50 value of 0.18mg/ml. In comparison, Khak Dam and Holland *CPL* Leave showed IC50 values of 0.24 mg/mL and 0.44 mg/mL, respectively (27,28).

The measurement of the free-radical-scavenging possibility of acetone and n-hexane extracts from *CPL var. RL* at different intensities was conducted via the DPPH process. The result demonstrated that the acetone extract showed limited activity with 52.8%, 53.1% and 53.8% inhibition, whereas the n-hexane extract revealed no activity, illustrated -8.5%, -9.0% and 9.2% inhibitions. In contrast, the Gallic Acid revealed significant inhibition of 95.0%, 95.3% and 95.6% at a concentration of 0.5mg/ml, as illustrated in the table. Furthermore, the acetone extract of CPL. var. RL exhibited an IC50±SEM value of 476.4±0.51µg/ml, whereas Gallic Acid demonstrated an IC50 value of 3.69µg/ml. Tukey's post-hoc test showed that the antioxidant activity of gallic acid was significantly greater compared to acetone extract ($p < 0.001$) and n-hexane extract ($p < 0.001$). Moreover, the acetone extract was more active than the n-hexane extract ($p < 0.001$). In a 2016 study by Asghar N et al., the anti-oxidant property assessed by means of the free radical scavenging showed that extracts from roots, bark, leaves and pulp had over 75.0% scavenging property. Specifically, pulp and leaves exhibited 84.9% and 80.9% inhibition of per-oxidation, correspondingly (29). Another study by Kumar SS et al., 2024 the free-radical-scavenging revealed the butanolic fraction of young-leaves 97±1.155% and seeds 96.33±0.8819% revealed the highest antioxidant potential. This was followed by mature leaves-81.33±1.856%, mature seeds-72.67±0.8819%, mature pulp-51±1.155%, young pulp-43.67±2.028%, young peel-34.33±1.202%, and mature peel-30±2.309% (30).These results emphasized the differing levels of antioxidant activity between the extracts and the standard drug, indicating a potential discrepancy in their effectiveness in scavenging-free-radicals.

CONCLUSION:

Our evaluation of antioxidant activity indicates that acetone extracts exhibited minimal activity, at the same time as n-hexane extracts demonstrated no measurable capacity for scavenging-free-radicals when compared to the Std-drug.

RECOMMENDATIONS:

Based on results, various recommendations can be suggested to better our interpretation and consumption of these extracts. Primarily, optimization of extraction techniques should be measured by assessing various methods to better the produce and potency of anti-oxidant compounds from *CPL var. RL*. In addition, examination with alternative solvents or extraction may lead to the detection of more potent extracts. Lastly, studying clinical-trials to assess their possible health benefits in humans could deliver appreciated understandings into their medicinal potential against oxidative-stress related illnesses or general-health improvement.

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Authors' Contribution

- **Concept & Design of Study, Data Collection**: Asadullah, Asif Ahmed,
- **Drafting**: Asadullah, Asif Ahmed, Muhammad Jabran Javaid Sidhu
- **Data Analysis:** Sana Shahzad, Naima Khalid, Anam Arshad
- **Critical Review**: Asadullah, Asif Ahmed, Muhammad Jabran Javaid Sidhu Anam Arshad

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