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ASSAY OF COMPREHENSIVE PHYTOCHEMISTRY AND EVALUATION OF IN-VITRO ANTIOXIDANT ACTIVITIY OF LEAF EXTRACT OF CARICA PAPAYA

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ABSTRACT

In India indigenous remedies have been used in treatments of various diseases. Moreover, phytochemical screening is an important step which leads to the isolation of new and novel compounds. Hence in the current study we aimed to assay comprehensive phytochemistry and evaluation of antioxidant activities of methanolic leaf extract of Carica papaya. Leaves of C. papaya was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. The in-vitro antioxidant activity of methanolic leaf extract of C. papaya was determined using DPPH free radical scavenging method. Results revealed that the IC_{50} values exhibited by methanolic leaf extract of C. papaya and standard ascorbic acid was found to be 21.52 µg/mL and 28.46 µg/mL respectively. The major phytochemicals found in methanolic leaf extract of C. papaya were found to be alkaloids, flavonoids, saponins, phenolic compounds, and tannins. Furthermore, methanolic leaf extract of C. papaya contains highest quantities i.e., 128.36 mg GAE/g of total phenolics as compared with total tannins (36.48 mg GAE/g) and total flavonoids (22.84 mg GAE/g). In conclusion, our study findings demonstrated antioxidant potential of methanolic leaf extract of C. papaya, and hence methanolic leaf extract of C. papaya could be explored in the development natural antioxidant drugs.

Keywords: Leaves, Carica papaya, Methanol, Antioxidant, Phenols, Flavonoids, Tannins

INTRODUCTION

Plant materials are used throughout the world as home remedies, over the counter drug products and raw substances for the pharmaceutical industry, cosmetics industry and represent a substantial proportion of the world drug market. Plant leaves with varied medicinal potential have known to remedial for human beings. These leaves of plants were selected as Indian traditional medicinal plants they are known to possess useful biological properties like antioxidants, anti-inflammatory, anticytotoxic, anticancer etc.[1]

Phytochemicals are naturally occurring in different parts of the medicinal plants that have defense mechanism and offer protection from a variety of ailments.[2] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents which produce definite physiological action on the human body and these bioactive substances include alkaloids, carbohydrates, terpenoids, steroids, flavonoids, tannins, etc.[3]

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Free radicals are continuously being produced in our body as a result of various metabolisms. Some amount of free radicals are very much necessary for body host defense system, signalling mechanism and in the induction of a mitogenic response.[2] But the persistence of these free radicals even after their activity results in deleterious effects. These free radicals act on important biomolecules like nucleic acids (mutations), lipids (membrane lipid peroxidation), proteins (oxidation) and carbohydrates resulting in various diseases. Over 70 degenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, depression, arthritis, cancer, ageing etc..) are caused due to free radicals.[4-6] Herbal medicines are the materials which are derived from one or more plant which possess some curative values to prevent human body from common diseases.[7] Free radical scavenging property can be removed completely by the antioxidants and maintains the balance in the body.[8]

Natural or herbal antibiotics are "smart" in a way that typical antibiotics are not. In addition, natural or herbal antibiotics have no side effects on the human body. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effect, easily available at the affordable price and sometime the only source of health care available to the poor.[9] Carica papaya is the sole species in the genus Carica of the plant family Caricaceae.[10] The papaya is a large tree with a single stem growing from 5 to 10 meters tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 centimeters diameter. The flowers are similar in shape to the flowers of the Plumeria, but are much smaller and wax-like. They appear on the axils of the leaves, maturing into the large 15–45 centimeters long (Figure 1).[11]

Figure 1. Showing Carica papaya plant

Previously published studies have well documented the usage of C. papaya leaf, seed, fruit and peel extracts owing to its antioxidant properties.[12-15] Hence in the current study we aimed to assay comprehensive phytochemistry and evaluation of antioxidant activities of leaf extract of C. papaya.

MATERIALS AND METHODS

Collection Leaves of C. papaya

C. papaya leaves were collected from local market in Bengaluru urban district, Karnataka, India. The C. papaya leaves were washed in tap water and then rinsed in sterile distilled water, and then shade dried at room temperature for one week. The dried leaves of C. papaya were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50g of dried and coarsely powdered leaves of C. papaya were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Qualitative Analysis

Qualitative analysis of methanolic leaf extract of C. papaya was carried out through phytochemical screening using standard procedures to detect phytoconstituents as described by Sofora,[16] Trease and Evans[17] and Harborne.[18]

Quantitative Analysis

Total phenolics

The concentration of total phenolics in methanolic leaf extract of C. papaya was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[19] The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in methanol solution and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Total Flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in methanolic leaf extract of C. papaya.[20] The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Tannins

The tannin concentration was determined in methanolic leaf extract of C. papaya following a modified version of the vanillin-HCl method.[21] The concentration of tannin was expressed in terms of mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Assay of Antioxidant Activity

The modified literature protocol of Blois was used for antioxidant assay.[22,23] Briefly 2, 2-diphenyl 1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1 picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. Scavenging of free radicals by DPPH as percent

radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation i.e., Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

RESULTS

The major phytochemicals found in methanolic leaf extract of C. papaya were found to be alkaloids, flavonoids, saponins, phenolic compounds, and tannins. Whereas, phytochemicals terpenoids were found to be absent in methanolic leaf extract of C. papaya (Table 1).

Table 1. Qualitative analysis of methanolic leaf extract of C. papaya

Phytochemical Components	Methanolic Leaf Extract of C. papaya
Alkaloids	+
Flavonoids	+
Saponins	+
Phenolic compounds	+
Tannins	+
Terpenoids	-

+: Present; -: Absent

The results of quantitative estimation of phytochemicals in methanolic leaf extract of C. papaya was represented in Table 2. Results revealed that methanolic leaf extract of C. papaya contains highest quantities i.e., 128.36 mg GAE/g of total phenolics as compared with total tannins (36.48 mg GAE/g) and total flavonoids (22.84 mg GAE/g).

Table 2. Quantitative estimation of phytochemicals in methanolic leaf extract of C. papaya

Phytochemical Components	Methanolic leaf Extract of C. papaya
Total phenolics	128.36 mg GAE/g
Total flavonoids	22.84 mg GAE/g
Tannins	36.48 mg GAE/g

Values were expressed as mean; n=3

The results of in-vitro antioxidant activities of standard (ascorbic acid) and methanolic leaf extract of C. papaya was represented in Table 3. Results depicted that the IC_{50} values exhibited by methanolic leaf extract of C. papaya and standard ascorbic acid was found to be 21.52 µg/mL and 28.46 µg/mL respectively (Table 3). These findings implied that methanolic leaf extract of C. papaya showed antioxidant activity at par with that of standard ascorbic acid.

Table 3. Antioxidant activities of methanolic leaf extracts of C. papaya

S. No.	Sample	IC_{50} (µg/mL)
1	Methanolic leaf Extract of C. papaya	21.52
2	Ascorbic acid	28.46

Values were expressed as mean; n=3

DISCUSSION

Due to the biologically advantageous effects of plant-based products resulting from the antioxidant activities of phenolic phytochemicals, active research on plant-based products has been driven in recent years. Because they have no harmful effects on people, and hence plant products are preferred to synthetic compounds in the treatment of diseases.[24] Therefore in the present study we aimed to assay comprehensive phytochemistry and evaluation of antioxidant activities of methanolic leaf extract of C. papaya.

DPPH is often used to determine free radical scavenging activity of natural compounds due to its stability as a radical. The presence of unpaired electron imparts a strong absorbance at 517 nm, giving the radical a purple color. With the exposure to antioxidants, it undergoes reduction, decreasing absorbance due to the formation of yellow colored anti-radical diphenyl picryl hydrazine (DPPH). The degree of colour change from purple to yellow is a measure of scavenging potential of the antioxidants in the extracts in terms of hydrogen donating ability.[24] In our study methanolic leaf extract of C. papaya showed antioxidant activity at par with that of standard ascorbic acid by DPPH assay method. Kadiri et al., reported that 1 mg/ml ethanolic papaya leaf extract demonstrated significant antioxidant properties by protecting DNA damage in lymphocytes.[25]

In our study quantitative estimation of phytochemicals in methanolic leaf extract of C. papaya revealed that methanolic leaf extract of C. papaya contains highest quantities i.e., 128.36 mg GAE/g of total phenolics as compared with total tannins (36.48 mg GAE/g) and total flavonoids (22.84 mg GAE/g). Scavenging of DPPH by methanolic leaf extract of C. papaya may be accredited to their phenolic compounds, flavonoids and tannins.[24]

Furthermore, literature reports evidenced that phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.[26] Vinson et al., conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables.[27] Flavonoids are versatile bioactive secondary metabolites present in almost all

plant species. Most representative family members include flavones, flavanes, flavonols, catechins, and anthocyanidins. Their antioxidant potential toward reactive oxygen species depends on structural characteristics such as the number and substitution pattern of hydroxyl groups and the extent at which these groups are glycosylated.[28]

CONCLUSION

In conclusion, our study findings demonstrated antioxidant potential of methanolic leaf extract of C. papaya, and it is accredited to secondary metabolites like phenolic compounds, flavonoids, and tannins present in it. Therefore, methanolic leaf extract of C. papaya could be exploited in the development natural antioxidant drugs.

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