INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHARMACOGNOSTIC STUDY OF *CARICA PAPAYA* LEAF EXTRACT AS INHIBITORS OF REACTIVE OXYGEN SPECIES

Ankit Nariya and Devendrasinh Jhala *
Cell biology Lab., Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad, India
*Corresponding Author Email: ddjhala@gmail.com

Article Received on: 19/01/17 Approved for publication: 20/02/17

DOI: 10.7897/2230-8407.080328

ABSTRACT

Natural antioxidants in plants, vegetables and fruits, such as alkaloids, flavonoids and phenols have been associated with the prevention of several degenerative mechanisms. The extraction of plant constituents is essential to isolate biologically active compounds. The identification of components is important to understand their role on the treatment of various anomalies. *Carica papaya* belonging to the Caricaceae family is an effective medicinal herb that is being used as a folk medicine for the treatment of various diseases throughout the world. The present study has been carried out to explore the preliminary phytochemicals and physicochemical analysis of 70% Methanolic extract of *Carica papaya* leaf. The physicochemical screening of carbohydrate, protein, fats and oils, alkaloids, tannins, saponins, steroids, glycosides and flavonoids was conducted. The tests were conducted in triplicate and quantitative determination of the various metabolites (i.e. Total Phenols, Flavonoids and Tannins) was done using respective analytical standards (Gallic acid, Quercetin and Tannic acid respectively). The total antioxidant activity was also evaluated using different models of free radical assay (i.e. DPPH, Superoxide and H_2O_2 scavenging assay). The phytochemical analysis revealed the presence of Carbohydrate, Proteins, Steroids, Triterpanoids, Flavonoids, Phenolic Compounds, Tannins, Alkaloids, Saponins and Fats in 70% Methanolic *Carica papaya* leaf extract. Enough content of phenols, flavonoids and tannins was also observed in quantitative analysis which was resulted into free radical scavenging properties in different assays. In conclusion *Carica papaya* leaf extracts are the reserve of important phytochemical leading to antioxidative supremacy. Present study can give support to develop quality standards using *Carica papaya* leaf and useful in drug development to mitigate various abnormal conditions.

Key words: *Carica papaya*, ROS, Phytochemicals, Methanolic extract

INTRODUCTION

Carica papaya belongs to the small family Caricaceae and was being used as a folk medicine in various treatments. *Carica papaya* commonly known as papaya in English, Papita in Hindi and Erandarkarkati in Sanskrit. There is always a soft main trunk and tufted leaves at the top in papaya plant. Plant is native to tropical America and was introduce in India in 16th Century. Besides the fruits being the fruits being edible, it has been long history and proof of being a very effective medicinal plant. Papaya leaf varies in sizes, shape in different maturity stages. Leaves spirally arranged, clustered near apex of trunk; petiole up to 1 m long, hollow, greenish or purplish-green; lamina orbicular, 25-75 cm in diameter, palmate, deeply 7 lobed, glabrous, prominently veined; lobes deeply and broadly toothed[1].

Papaya contains a broad spectrum of phytochemicals including enzymes (in the latex), carotenoids (in fruits and seeds), alkaloids (in leaves), phenolics (in fruits, leaves, and shoots), and glucosinolates (in seeds and fruits). Papaya leaf has a numberless of benefits. Previous studies have shown that papaya possesses activities against protozoan, bacterial, fungal growth, inflammation, hypertension, wound, tumor, free radicals[2]. In India papaya leaves are being used specifically for fever, beriberi, asthma, colic[3]. Also, the young leaves of papaya are eaten like spinach in some part of Asia. Moreover, juice of papaya leaf is also used to increase platelets and white blood cells for normal clotting. The leaves used into tea for malaria treatment and dried and cured leaves were also used as cigar and smoked by asthmatic persons[4]. Beside these it is also being used for relieve nausea, ease menstrual pain and tenderization of meat[5].

Oxidative stress and *Carica papaya*

A free radical is harmful because in search for a pairing electron, the free radical takes one electron from a stable molecule, in turn the stable one becomes a free radical and the resulting chain reaction can injure tissues and impair their functions Many scientific investigations have been conducted to evaluate the biological activities of various parts of *Carica papaya*, including seeds, root, leaves, fruits latex etc. Several scientific studies validate many traditional uses by demonstrating therapeutic activities *of Carica papaya* leaves[3, 6]. Various beneficial properties of papaya mostly subject to rich source of antioxidants and activities of different metabolites obtainable from *Carica papaya* organs especially from leaves. It was significant that papaya leaf is rich source of antioxidants so the higher its possibility to reduce oxidative stress by scavenging free radicals[7]. Thus, *Carica papaya* leaf extracts may act as a synergistic therapeutic dietary supplement in patients with oxidative stress related diseases[8].

Carica papaya leaves contain many active components that can increase the total antioxidant activity in blood. Leaves extracts contains folic acid, vitamin B12, vitamins A, vitamins C, alkaloids, saponins, glycosides, tannins, and flavonoids with anti-inflammatory, anticancer activity and protection against the oxidative damage[9]. It also found to have anti-bacterial, anti-tumor and immunomodulatory activities[10].

The involvement of oxidative stress mechanisms in several biological and pathological processes including ageing, cancer, cardiovascular and neurodegenerative diseases has continued to

fuel suggestions that processes can potentially be modulated by treatment with free-radical scavengers and antioxidant like *Carica papaya* leaf extracts. The safety and antioxidative stress potential of papaya juice is found to be comparable to the standard antioxidant compound alpha tocopherol. The preparation containing yeast fermented papaya as one of the constituent has antioxidant actions and that it may be prophylactic food against age related and neurological diseases associated with free radicals. Bacteriostatic activity of papaya could be correlated to its scavenging action on superoxide and hydroxyl radicals. Bio-catalyzer, which contains yeast fermented papaya, may be useful as health foods against neural lipid peroxidation, traumatic epilepsy and ageing. Consumptions papaya fruits reduce oxidative stress and alter lipid profile. Thus, it could reduce the risk of disease caused by free radical activities and high cholesterol in blood. Papaya has many phenolic groups which may scavenge free radicals effectively[3, 5].

MATERIALS AND METHODS

Collection of plant Sample and Drying of Leaf

Young leaves of *Carica papaya* were collected from Veraval region, Gujarat, authenticated by Department of Botany, School of Sciences, Gujarat University, Ahmedabad – 09. The leaves of *Carica papaya* were shade dried at room temperature. After complete drying, the dried leaves were then grounded in coarse powder using grinding machine. The coarse powder was then stored in sterile airtight container at room temperature away from moisture for further study.

70% Methanolic (MeOH) Extract Preparation

The known amount of dried powder of papaya was mixed with 300 ml of 70% MeOH. The mixture was stirred on stirrer for 72 hours. Further the mixture was filtered 2 – 3 times in sterile condition. Subsequently the filtrate was evaporated at 37 – 40 °C in incubator. After evaporation the dried content of filtrate was used as 70% MeOH extract for further analysis. The percentage yield and loss on drying was calculated using given formula[11]:

Percentage Yield

$$\text{Percentage Yield } \% = \frac{\text{Weight of product after evaporation}}{\text{Weight of poweder used}} \text{ X } 100$$

Loss on Drying

$$\text{Loss on drying } \% = \frac{\text{Extrative Value}}{\text{Total weight used}} \text{ X } 100$$

Phytochemicals Analysis

Phytochemicals were analysis for the extracts particularly to ascertain the presence of different bioactive components present in 70% MeOH *Carica papaya* leaf extract. The presence of alkaloids, saponins, tannins, flavonoids, steroidal terpenes and carbohydrates were determined, as described by the method of Harborne[12].

Test for Carbohydrates: Molisch, Fehling's, Benedict's and Barfoed test was performed to determine the presence of Carbohydrates.

Test for Proteins: Biuret, Millon's and Xanthoprotein test was performed to determine the presence of Protein.

Test for Steroids and Triterpanoids: Salkowski and Liebermann-burchard test was performed to determine the presence of Steroids and Triterpanoids.

Test for Amino Acids: Ninhydrin test was performed to determine the presence of Amino Acids.

Test for Flavonoids: Shinoda, Lead acetate and NaOH test was performed to determine the presence of Flavonoids.

Test for Tannin and Phenolic compounds: $FeCl_3$, Lead acetate, dil. HNO_3, Acetic Acid and Iodine test was performed to determine the presence of tannin and Phenolic compounds.

Test for Alkaloids: Dragendorff's, Mayer's, Wagner's and Hager's test was performed to determine the presence of Alkaloids.

Test for Saponins: Foam test was performed to determine the presence of Saponins.

Test for Fats To determine fats few drops of sample was taken on blotting paper and checked for oily surface of blotting paper. The oily surface of blotting paper determines the presence of fats.

Quantitative estimation of Phytoconstituents

The total phenolic content of 70% MeOH *Carica papaya* leaf extract was estimated according to the method of Singleton and co-workers[13]. The determination of flavonoids and tannins was performed according to the method of Harborne[12].

Free radical scavenging Activity

DPPH Scavenging activity

DPPH free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the method of MacDonald-Wicks and co-workers[14] for various concentration 70% MeOH extract of CP leaves (10 to 100 µg).

Superoxide scavenging activity

Superoxide free radical was generated by xanthine-xanthine oxidase and detected by the NBT reduction using the method of Alves and co-worker[15] for various concentration 70% MeOH extract of CP leaves (10 to 100 µg).

Hydrogen peroxide-scavenging activity

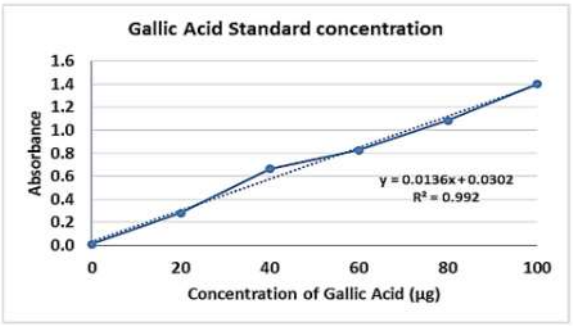
Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch and co-workers[16] for various concentration 70% MeOH extract of CP leaves (10 to 100 µg).

Calculation

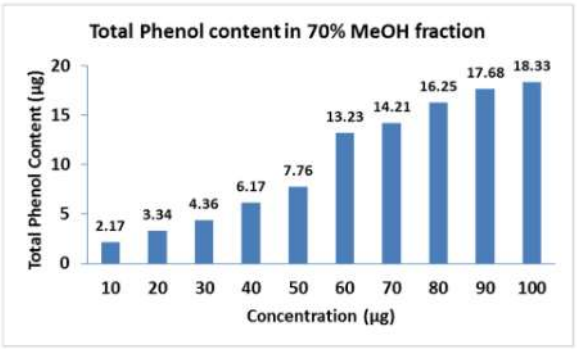
$$\text{Scavenging Activity } \% = \frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \text{ X } 100$$

Table 1: Physicochemical and Phytochemical analysis of 70% MeOH extract of *Carica papaya* leaf

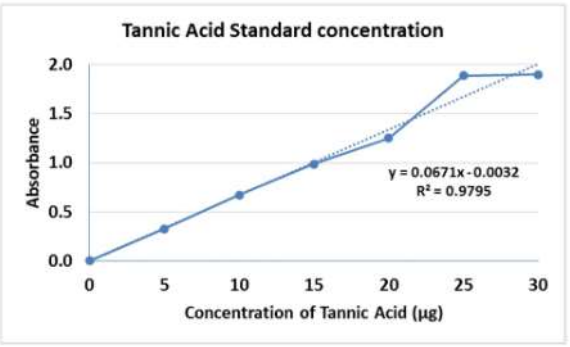
Test parameters	70% MeOH Extract
Percentage Yield	32.50 %
Loss on drying (%)	2.22 %
pH	5
Day light Colour of Extract	Dark Brown
Carbohydrate	+
Proteins	+
Steroids & Triterpanoids	+
Amino Acid	-
Flavonoids	+
Phenolic Compounds	+
Tannins	+
Alkaloids	+
Saponins	+
Foaming index (%)	<1
Fats	+



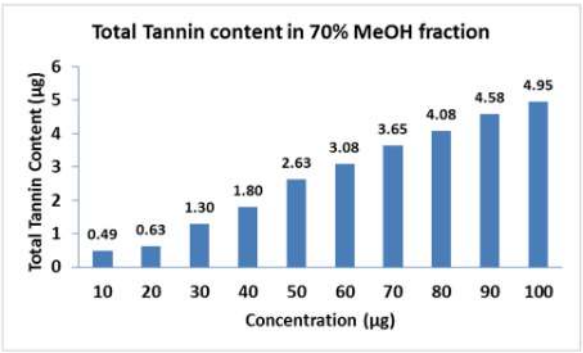
Graph 1: Graph of absorbance v/s various known concentration of Gallic acid



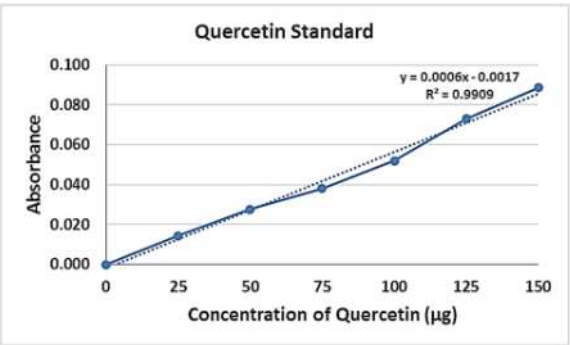
Graph 2: Total Phenolic content at various concentration of 70% MeOH CP leaf Extract



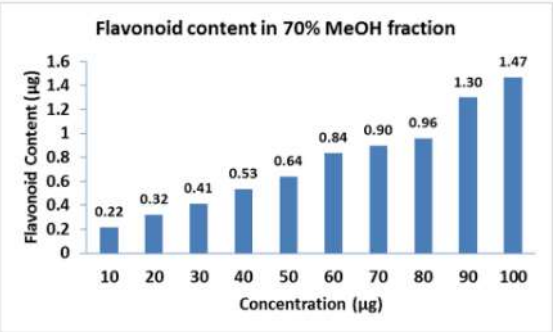
Graph 3: Graph of absorbance v/s various known concentration of Tannic acid



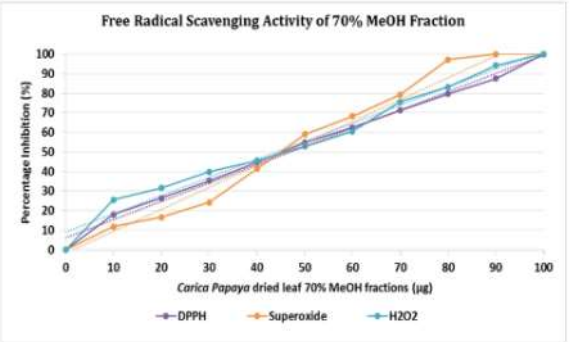
Graph 4: Total Tannin content at various concentration of 70% MeOH CP leaf Extract



Graph 5: Graph of absorbance v/s various known concentration of Quercetin



Graph 6: Total Flavonoid content at various concentration of 70% MeOH CP leaf Extract



Graph 7: Free radical scavenging activity in various concentration of 70% MeOH CP leaf Extract

RESULTS

Physicochemical and Phytochemical analysis of 70% MeOH extract of *Carica papaya* Leaf

In this study 32.50% extractive value was obtain from total CP leaf used. There was 2.22% loss of total extractive value in whole extraction procedure. Present study also showed that the day light colour of the 70% MeOH extract of CP leaf was dark green with pH 5. The study revealed that there was presence of various phytochemicals like carbohydrate, proteins, steroids, triterpenoids, flavonoids, tannins, phenolic compounds, alkaloids, saponins and fats in the 70% MeOH extract of CP leaf. Only ninhydrin test for amino acids showed negative results and foaming index was less than 1 (Table 1).

Total Phenolic content (TPC), Total Tannin content (TTC) and Total Flavonoid content (TFC)

The TPC was determined in comparison with standard Gallic acid (Graph 1) and the results were expressed in terms of µg/ml of extract equivalent to µg of gallic acid. It ranged from 2.17 to 18.33 µg in concentration between 10 to 100 µg (Graph 2).
This study showed that there was increased TPC, TTC and TFC in accordance to extract concentrations (10 to 100 µg).
The TTC was determined in comparison with standard Tannic acid (Graph 3) and the results were expressed in terms of µg/ml of extract equivalent to µg of tannic acid. It ranged from 0.49 to 4.95 µg/ml in concentration between 10-100 µg (Graph 4).
The TFC was determined in comparison with standard Quercetin (Graph 5) and the results were expressed in terms of µg/ml of extract equivalent to µg of Quercetin. It ranged from 0.22 to 1.47 µg/ml in concentration between 10-100 µg (Graph 6).

Free radical scavenging activity

Present study showed that there was concentration dependent inhibition of DPPH, Superoxide and Hydrogen peroxide activity (Graph 7). This inhibition rapidly increased (30 to 100%) by increased in the concentration from 10 to 100 µg of 70% MeOH CP leaf extract. Data showed that there was 17.98% to 100% inhibition of DPPH; 11.86% to 100% inhibition of Superoxide and 25.26% to 100% inhibition of Hydrogen peroxide was obtained by 10 to 100 µg concentration of 70% MeOH CP leaf extract (Graph 7) So, 100% inhibition of DPPH, Superoxide and Hydrogen peroxide was observed at the concentration of 100 µg/ml of CP leaf extract. The concentration dependant increase in the radical scavenging activity of this extract showed that high amount of radical scavengers were present in the 70% MeOH CP leaf extract.

DISCUSSION

Herbal medicines are valuable and readily available resources for primary health care system. Undoubtedly the plant kingdom still holds many species containing substances of medicinal values that are yet to be discovered. The solubility of antioxidant compounds in solvent was found to have a significant effect on the recovery of compounds at the time of extraction. Thus, the polarity of solvents has an indirect function in the extraction process, because it can raise the solubility of antioxidant compounds[17]. Frequently used solvents for antioxidant compound extraction (from fresh fruits/plants) include acetone, ethanol, methanol, chloroform, ethyl acetate, etc.[17,18]. In the present study, the extraction efficiency (32.50%) was higher as there is combination of water and methanol (3:7) was used as a solvent (i.e. 70% MeOH). It is clear that the addition of some amount of water in different solvent enhances the extraction efficiency as evidenced in this study which is also in accordance with Rostagno and co-workers[19]. In the present study carbohydrate, proteins, steroids, triterpanoids, flavonoids, tannins, phenolic compounds, alkaloids, saponins and fats were observed using different phytochemical analysis study (Table 1). In support to this study Ali and coworkers[20] also revealed that, methanol solvent was most effective in extracting phenolic components.

The result also indicates that the papaya leaves have high total phenolic content (TPC) that may provide good sources of dietary antioxidant. Hence, it is obvious that TPC present in the extract along with other phytochemicals have strong effect against the free radical activity. Khamsah and co-workers[21] found that free radicals scavenging activity is not only due to the phenolic content itself, but with other various antioxidant compounds were also involved. In addition to this, TPC does not incorporate necessarily to all the antioxidants that may present in the extracts. Phenolic compounds along with other important components are widely distributed in plants[22], which have gained greatly attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health. The other compounds were also present in adequate amount as described in present study and it was supported by other studies also[21-24]. Along with TPC papaya leaf extract was showed presence of various component especially alkaloids, flavonoids and tannins. As increase in concentration of 70% MeOH *Carica papaya* leaf extract, the phycoconstituents' (Phenolics, Flavonoids and Tannins) concentration was also increase. Biological systems can produce hydrogen peroxide, superoxide free radicals which is harmful to human body. Hydrogen peroxide itself is not very active, but it can sometimes be toxic to cells, since it may give rise to hydroxyl radicals inside the cells. The idea of a single measurement of total antioxidant capacity is insufficient. There are various antioxidant activity methods have been used to evaluate and compare the antioxidant activities of plants. In this study, free radical scavenging activity was determined for the 70% MeOH CP leaf extract. Being a stable free radical the DPPH assay along with Superoxide and Hydrogen peroxide scavenging assay are simple and rapid methods which were frequently used to evaluate the ability of antioxidants to scavenge free radicals. It gives reliable information concerning the antioxidant ability of the tested compounds to act as free radical scavengers or hydrogen donors[25]. Generally, the mechanisms of phenolic compounds along with other components (i.e. alkaloids, tannins etc.) for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. Phytoconstituents may act against free radical through the reaction with the oxygen radicals, superoxide anion radicals and hydroxyl radicals as evidenced in this study. In the present study scavenging effects increased with increase in the concentration of extract. The results suggested that the extracts displayed scavenging effect on DPPH, superoxide radical generation and H_2O_2 that could prevent ameliorate oxidative damage. There was difference in concentrations which was required for free radical-scavenging activities in different assays. More concentration of CP extract was required to scavenge DPPH while lesser concentration to scavenge superoxide and hydrogen peroxide as observed in this study. This showed that the CP leaves exhibit a strong free radical scavenging activity due to the phytochemicals especially phenolics constitute a major group of compounds that act as primary antioxidant. The study of Hatano and co-workers[25] also support these findings. So, the present study confirms the protective effects of 70% MeOH *Carica papaya* leaf extract against free radicals.

CONCLUSION

The study suggests that 70% MeOH *Carica papaya* leaf extract possess a wide range of pharmacological activities resulted in antioxidative action which can counteract the oxidative damage.

Due to the antioxidant activities exhibited by the 70% MeOH CP leaf extract, it could be considered a natural herbal source that can be used in food and pharmaceutical industries. However, further studies required to obtain purified compounds that may be more effective and responsible for the activities observed from the tested leaves.

REFERENCES

1. Orwa CA, Mutua R, Kindt R, Jamnadass, Anthony S. Agroforestree Database: A tree reference and selection guide version 4.0; 2009 [cited 2017 Feb 10]. Available from: http://www.worldagroforestry.org/output/agroforestree-database
2. Lim T. Edible medicinal and non-medicinal plants. Fruits; 1st ed. Springer Science and Business Media(NY): Springer Netherlands; 2012.
3. Krishna KL, Paridhavi M, Patel JA. Review on nutritional, medicinal and pharmacological properties of papaya (*Carica papaya* Linn.). Natural Product Radiance 2008; 7:364-373.
4. Vijay Y, Pradeep KG, Chetan SC, Anju G, Bhupendra V. Carica papaya Linn: An Overview. International Journal of Herbal Medicine. 2015; 2 Suppl 5:01-08.
5. Aravind G, Debjit B, Duraivel S, Harish G. Traditional and Medicinal Uses of *Carica papaya*. Journal of Medicinal Plants Studies 2013; 1 Suppl 1:7-15.
6. Jaiswal P, Kumar P, Singh VK, Singh DK. *Carica papaya* Linn: a potential source for various health problems. Journal of Pharmacy Research 2010; 3 Suppl 5:998–1003.
7. Imaga NA, Gbenle GO, Okochi VI, et al. Phytochemical and antioxidant nutrient constituents of *Carica papaya* and parquetina nigrescens extracts. Scientific Research and Essays 2010; 5 Suppl 16:2201–2205.
8. Elisa P, Majdi D, Stefania M, Cristian V, Luciana D. Administration Dependent Antioxidant Effect of *Carica papaya* Seeds Water Extract. Evidence-Based Complementary and Alternative Medicine 2014; 281508.
9. Indran M, Mahmood AA, Kuppusamy UR. Protective effect of *Carica papaya* L leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats. West Indian Medical Journal 2008; 57 Suppl 4:323–326.
10. Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. Journal of Ethnopharmacology 2010; 127 Suppl 3:760–767.
11. Varisha A, Ansari SH, Naquvi KJ, Arora P, Ahmad A. Development of quality standards of *Carica papaya* Linn. Leaves, Der Pharmacia Lettre 2013; 5 Suppl 2:370-376.
12. Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. Champman and Hall, London 1984; 014-188.
13. Singleton VR, Orthifeer R, Lamuela-Raventos RM. Analysis of Total phenols and other oxidation subs tracts and antioxidants by means of Folin-Ciocalteus reagent method in Enzymology 1999; 299:152-178.
14. MacDonald-Wicks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant capacity in vitro. A review. Journal of the Science of Food and Agriculture 2006; 86 Suppl 13:2046.
15. Alves CQ, David JM, David JP, Baia MV, Aquian RM. Method for determination of in vitro antioxidants activity for extracts and organic compounds. Quimica Nova 2010; 33:2202-2210.
16. Ruch RT, Cheng SJ, Klaunig JE. Spin trapping of superoxide and hydroxyl radicals. Methods in enzymology 1984; 105:98-209.
17. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 2009; 115:785–788.
18. Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner KL, Baldwin EA. Total antioxidant activity and fiber content of select Floridagrown tropical fruits. Journal of Agricultural and Food Chemistry 2006; 54 Suppl 19:7355-7363.
19. Rostagno MA, Palma M, Barroso CG. Ultrasound-assisted extraction of soy isoflavones. Journal of Chromatography 2003; 1012:119-128.
20. Ali SG, Rab A, Khan NU, Nawab K. Enhanced proline synthesis may determine resistance to salt stress in tomato cultivars. Pakistan Journal of Botany 2011; 43 Suppl 6:2707-2710.
21. Khamsah SM, Akowah G, Zhari I. Antioxidant activity and phenolic content of Orthosiphon stamineus benth from different geographical origin. Journal of Sustainability Science & Management 2006; 1:14–20.
22. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit. Rev. Food Science and Nutrition 1992; 32 Suppl 1:67-103.
23. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Mehrotra S, Palpu P. Studies on the Antioxidant Activities of Desmodium gangeticum. Biological and Pharmaceutical Bulletin 2003; 206:1424-1427.
24. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16:144-158.
25. Hatano TR, Edamatsu M, Hiramatsu A, et al. Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl radical. Chemical and pharmaceutical bulletin 1989; 37:2016-2021.

Cite this article as:

Ankit Nariya and Devendrasinh Jhala. Pharmacognostic study of *Carica papaya* leaf extract as inhibitors of reactive oxygen species. Int. Res. J. Pharm. 2017;8(3):13-17 http://dx.doi.org/10.7897/2230-8407.080328

Source of support: Gujarat State Biotechnology Mission, Gandhinagar, Conflict of interest: None Declared