Research article

Studies on antioxidant potential and total phenolic contents of dried powder and pulp of *raw and ripe Carica papaya* fruit

Bhardwaj Madhvi[1], Mahajan Babita[1], Kumar Ankush[2], Parcha Versha[3]

[1]Department of Chemistry, G.G.M. Science College, Jammu, J&K, India
[2]P.G. Department of Chemistry, Government (PG) Degree College, Bhaderwah, J&K, India
[3]Department of Pharmaceutical Chemistry & Chemistry, Dolphin (PG) Institute of Biomedical & Natural Sciences, Manduwala, Dehradun, India

(Received: December 2022 Revised: March 2023 Accepted: April 2023)

Correspondence author: **Versha Parcha**. Email: vershaparcha@gmail.com

ABSTRACT

Introduction and Aim: Antioxidants are frequently used as active ingredients in anti-aging supplements with the intention of preserving health and preventing diseases. However, recent research has concentrated on conventional therapies that have a long history of successfully treating several diseases. *C. papaya* is a good alternative and exhibits significant antioxidant, diuretic, antihyperglycemic, anticancer, analgesic, and depressive properties from a key plant constituent. In this study, total phenolic content, and antioxidant potential of raw and ripe *Carica papaya* were examined.

Materials and Methods: C. Samples of raw and ripe *Carica papaya* were collected from the local market of Dehradun. Dried powder and fresh pulp of the raw and ripe *Carica papaya* was individually subjected to phytochemical test principles. Total phenolic contents were estimated using the Folin-Ciocalteu and antioxidant potential using DPPH and ferric chloride reducing power assay method.

Results: The fresh pulp and powder of ripe *Carica papaya* showed high content of phenolics in fresh ripe pulp, fresh raw pulp, dried ripe powder and raw dried powder as 200, 195,185 7 mg/100 g respectively with an antioxidant activity DPPH radical scavenging activity, 80.2, and 75. .76.4 and 74.5 respectively which is comparable to butylated hydroxy anisole (BHA) and diphenhydramine (DPPH) radical scavenging activity.

Conclusion: The fresh pulp and powder of ripe *Carica papaya* showed wide antioxidant activity (DPPH) radical scavenging activity. Hence, it can be effectively used as an organic substitute for chemical compounds (BHA, BHT) which have a lot of harmful side effects. Moreover, phytochemical studies of *Carica papaya* have shown the presence of phenolic compounds well justified by estimation of content which are responsible for its potent antioxidant activity.

Keywords: *Carica papaya*; antioxidant; phenolic content; radical scavenging; bioactive compound.

INTRODUCTION

The changing lifestyle and environmental conditions have predisposed common man towards numerous diseases. Many disorders involve oxidative damage via arthritis, Parkinson's, heart attack, AIDS, stroke, cancer, cataract, stress, and a long list of degenerative disorders including aging (1,2). At present, O_2 is being viewed as playing a lead role in the generation of Reactive Intermediates, thereby causing cellular damage. Oxidative damage generates Reactive O_2 species (ROS) N_2 species which mainly include free Radicals which are chemical entities possessing a single electron. Free radicals are manageable by the body, but when they are produced in excess, damage may result. Thus, numerous therapeutic approaches that incorporate the use of antioxidants (3) are available to address this issue.

Inhibiting chemical reactions involving oxygen is what antioxidant molecules or compounds do. Oxidation reactions, which harm cells in both humans and animals, are among these reactions. When free radicals cause harm, antioxidants stop it and fix it. When used as food additives, antioxidants have drawn a lot of interest. Antioxidants are frequently used in food products, both artificial and natural. For the body to maintain its natural health, natural antioxidants like lycopene, vitamin E, A & C, etc., are crucial. Natural antioxidants, which stop other molecules from oxidizing, are mostly found in the fresh juice and vegetables we eat. The use of natural antioxidants has been expanding due to the safety and ecological responsibility of these substances, as well as the increasing constraints on the use of synthetic antioxidants and increased public awareness of health issues. The use of some common synthetic antioxidants has generated debate, including (BHA) butylated hydroxy anisole and (BHT) butylated hydroxytoluene. In India and Romania, the use of BHT has already been outlawed after tests revealed metabolic stress. According to some studies, BHT combined with fats interfered with the liver's capacity to detoxify. BHT and BHA slow down rancidity, which is present in cereals, snacks, instant potatoes, and cosmetics (4). Rancidity is caused by oxidation of fats and oils. In this regard, C. papaya is a good substitute due to its powerful antioxidant , diuretic, anticancer, antihyperglycemic, analgesic, and depressive properties, which are all shown to be produced by a significant component of the plant. (5), (6). A genus of unbranched, small trees with rapid growth that are native to tropical America and have broad distribution throughout the region is called Carica Linn. (*Caricaceae*). There are four species in total, including *Carica papaya*, Linn. The species known best as "papaya" is the most popular and widely cultivated. It is cultivated almost everywhere in the subtropical zone for its mouthwatering fruits as well as to provide industrial papain, an enzyme with potent proteolytic activity that has applications in the pharmaceutical, cosmetic, and textile industries. The papaya tree is a small, fast-growing, single-stemmed tree that grows to a height of 2 to 10 metres. It has a smooth, void, ovoid, straight stem with enormous leaves and inflorescences that gives it a rough appearance. Papaya's alkaloid carpaine has been used as a diuretic and a stimulant to the heart (7,8).Here, we discuss the antioxidant potential of fresh pulp and dried powder made from raw and ripe C. papayas. Further investigations on the phytochemistry of the papaya plant have shown that it contains phenolic substances, which are what give the fruit its potent antioxidant properties.

MATERIALS AND METHODS

Collection of botanical material

Samples of raw and ripe *C. papaya* were collected from the local market. Cut into small pieces after the fruit's skin was peeled off. A 40°C air oven was then used to dry it. To produce the powdered dried fruit, the dried fruit was ground in a mixer. To obtain the fruit pulp, some of the raw and ripe fresh fruit was processed into a paste in a grinder mixture.

Qualitative phytochemical tests

Dried powder and fresh pulp of the raw and ripe *Carica papaya* was individually subjected to phytochemical tests (Table 1).

Antioxidant activity

DPPH radical scavenging assay

Fresh juice and powder were tested spectrophotometrically for their ability to scavenge free radicals against stable DPPH (2,2-diphenyl-2-picrylhydrazyl). DPPH is reduced when it interacts with an antioxidant substance that can donate hydrogen. Using a UV visible light spectrophotometer, the transition from purple to yellow was measured at 517 nm. The slightly modified method (9, 10) was used to measure the radical scavenging activity of fresh juice and powder. Methanol was used to create the extract solution. Even after treating the solution for 15 minutes in an ultrasonic bath, ethyl acetate and the aqueous extract were only partially soluble in methanol; as a result, they were filtered, and only the soluble portion was then examined. Fresh solution of DPPH in methanol was prepared each day. Before measuring absorption, the extract solutions and DPPH solution were combined in the ratio of 3:1 and kept in the dark for 30 minutes at room temperature. The experiment was carried out a total of three times. The following formula was used to determine the amount of radical scavenging activity:

(A_B-A_A/A_B) % Inhibition×100
A_B = Absorption of the Blank Sample in this case
A_A is the tested sample's absorption.

Ferric chloride reducing power assay

A quick and easy screening technique for determining antioxidant potential is the assay for lowering power. According to Banerjee procedure (11), the reducing power of the juice and powder was assessed. Tannic acid and gallic acid were employed as a standard. The reagents used were 0.2 M Sodium phosphate buffer, pH 6.6, 1%w/v Ferricyanide, 10% w/v Trichloroacetic Acid, 0.1%w/v Ferric Chloride. Juice and powder were combined with 5.0 ml of potassium ferricyanide and 5.0 ml of sodium phosphate buffer at concentrations ranging from 1.0 to 5.0 mg/ml of water. 5.0 ml of trichloroacetic acid were added after the mixture had been incubated at 50°C for 30 min. The mixture was then centrifuged at 980Xg for 10 min. at 50°C in a refrigerator-based centrifuge (VHM-17L Kokusan Denki, Tokyo, Japan). One ml of ferric chloride was added after the 5.0ml of the upper layer of the solutions were diluted with 5.0 ml of distilled water. Using a spectrophotometer (U-2001, Hitachi Instruments Inc.), absorbance was measured at 700 nm.

Increase in reducing power (%) = (A_T-A_B/A_B) ×100;
Where, A_B =Absorbance of the Blank Sample
A_T= Absorbance of Tested Sample

Total phenolic content (12)

Total phenolic content (TPC) was determined using the modified Singleton and Rossi technique (13). Each freeze-dried sample (200 mg) was extracted with 2 ml of 80% methanol over a two-hour period at room temperature in an orbital shaker set to 200 rpm. The mixture was then centrifuged for 15 minutes at 1000 rpm. Following a 10-fold dilution with distilled water, an aliquot of the supernatant was combined with 1.5 ml of Folin-Ciocalteu reagent and left to stand at room temperature. 1.5 ml of a 6% (w/v) sodium bicarbonate solution was added to the mixture after 5 minutes. After 90 minutes, the amount of light absorbed was determined spectrophotometrically at 725 nm. The standard calibration curve of gallic acid in 80% methanol (0.01 to 0.05 mg/ml) was plotted. The findings were expressed as small-cap Gallic acid equivalents (GAE) in mg per 100 g sample extracts.

RESULTS

Qualitative phytochemical analysis

The dried powder and fresh pulp of ripe and raw C. papaya were subjected to phytochemical analysis. The results of the various tests conducted indicated that phenols, saponins, terpenoids, and flavonoids were present in addition to carbohydrates. Table 1 displays the findings of the different analyses.

Antioxidant activity

DPPH radical scavenging activity

When an electron or a hydrogen atom is accepted, DPPH creates a stable molecule that is typically used to test the ability of chemical compounds, juices, and plants to scavenge free radicals.

The technique relies on the formation of the non-radical form of DPPH, which leads to the reduction of an alcoholic DPPH radical solution in the presence of an antioxidant that donates hydrogen. After a certain amount of time, the amount of DPPH still presents as measured corresponds inversely to the antioxidants' capacity to scavenge radicals.

The fresh pulp and powder of *C. papaya* demonstrated very strong DPPH radical scavenging action, according to the findings. BHA, which displayed the highest percentage of free radical scavenging activity, was employed as a positive control. i.e., 87.2% at a council of 5 mg/ml. Both fresh pulp and powder respectively ripe *C. papaya* showed a comparable radical scavenging activity i.e., 86% and 83.3% at concentrations of 5.0 mg/ml. Additionally, as the fresh pulp and dry powder concentration was raised, this free radical scavenging activity (% inhibition) also increased. Also, the raw C. papaya showed somewhat reduced activity than the ripe one, but the activity was still comparable viz 80.5% and 75.5% for the fresh pulp and dried powder. Figs. 1 and 2 display the findings of this analysis.

Table 1: The results of phytochemical analysis

S.No	Tests performed	Ripe powder	Raw powder	Ripe pulp	Raw pulp
1.	**Carbohydrates**	+ +	+ +	+ +	+ +
a.	Molisch	+ +	+ +	+ +	+ +
b.	Benedict’s	+ +	+ +	+ +	+ +
c.	Fehling’s	+ +	+ +	+ +	+ +
d.	Seliwanoff’s	+ +	+ +	+ +	+ +
e.	Barford’s	+ +	+ +	+ +	+ +
2.	**Proteins**				
a.	Million’s	- -	- -	- -	- -
b.	Biuret	- -	- -	- -	- -
c.	Ninhydrin	- -	- -	- -	- -
3.	**Phenols**	+ +	+ +	+ +	+ +
4.	**Saponins**	+ +	+ +	+ +	+ +
5.	**Terpenoids**	+ +	+ +	+ +	+ +
6.	**Flavonoids**				
a.	Ferric Chloride	+ +	+ +	+ +	+ +
b.	Vanillin HCl	+ +	+ +	+ +	+ +
c.	Zn-HCl Reduction	+ +	+ +	+ +	+ +

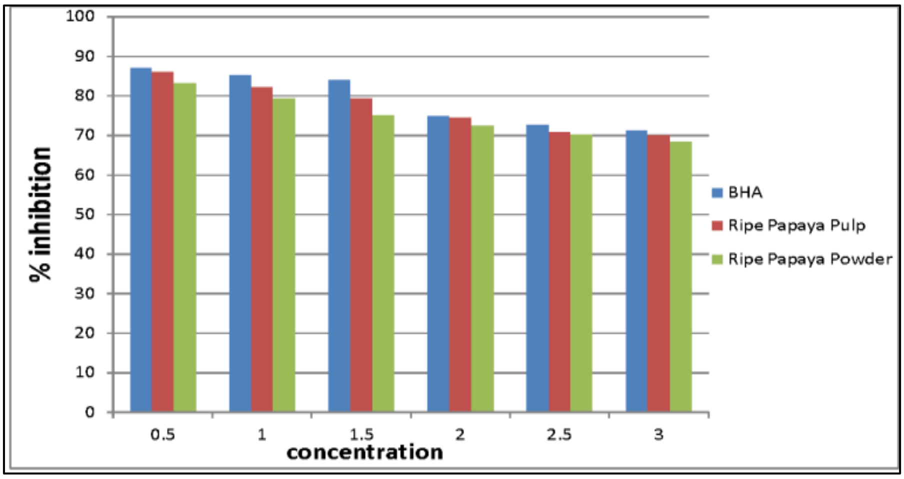

Fig. 1: % Inhibition of DPPH radical scavenging activity of ripe *C. papaya* fresh pulp & dried powder in comparison to the standard BHA

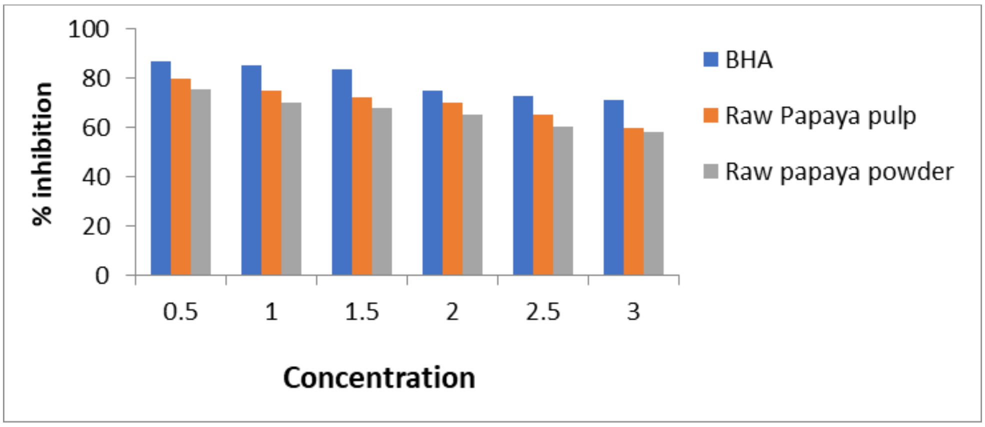

Fig. 2: % inhibition of raw *C. papaya's* ability to scavenge DPPH radicals Fresh pulp & dried powder in comparison to the standard BHA

Ferric chloride reducing power assay

A quick and easy screening method to determine the antioxidant potential is the reducing power test. Positive controls included ascorbic acid. With a modest alteration, the diminishing efficacy of the fresh pulp and powder of *C. papaya* was determined (14). The fresh pulp and dried powder of ripe and raw *Carica papayas* were tested for their ability to reduce various substances over the concentration range of 1.0-5.0 mg/ml. It was observed that the activity of all the samples increased as the concentration was raised.

The results of this analysis are solved in Fig. 3 and 4 and ripe and raw *C. papaya* respectively. As can be seen from the Figures, 5 mg/ml of ripe and dried powder assay of 80.2% and 76.4% respectively which was in good comparison to the Std. ascorbic acid (85%). Further, the raw fresh pulp & dried powder also showed good activity (i.e., 75% and 74.5% respectively).

Total amount of phenolics

Polyphenols are one of the primary groups responsible for the antioxidant activity of *C. papaya* (15-17). Phytochemical studies have the presence of polyphenols. We further carried out this analysis to estimate quantitatively as Gallic Acid equivalent (GAE). The polyphenols as mg/100 g of the sample. The results are shown in Table 2 and Fig. 5.

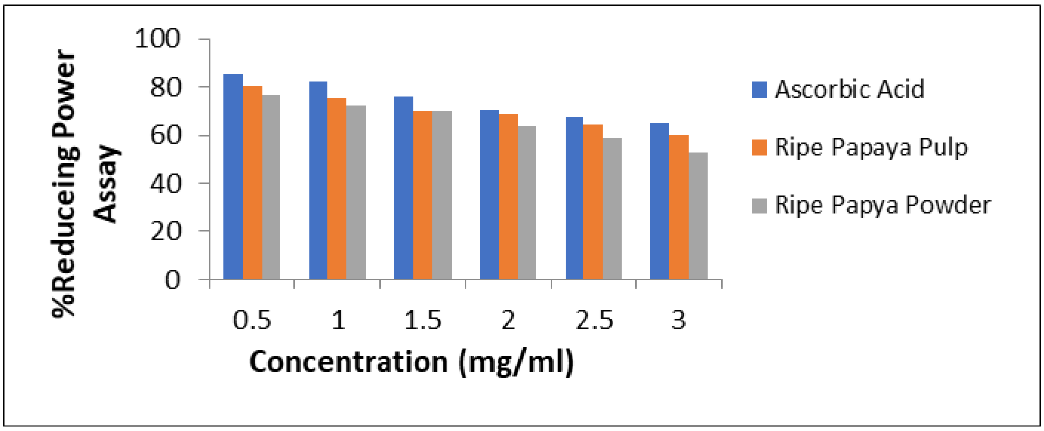

Fig. 3: % Inhibition of Ferric Chloride reducing power assay of Ripe *C. papaya* Fresh pulp & dried powder

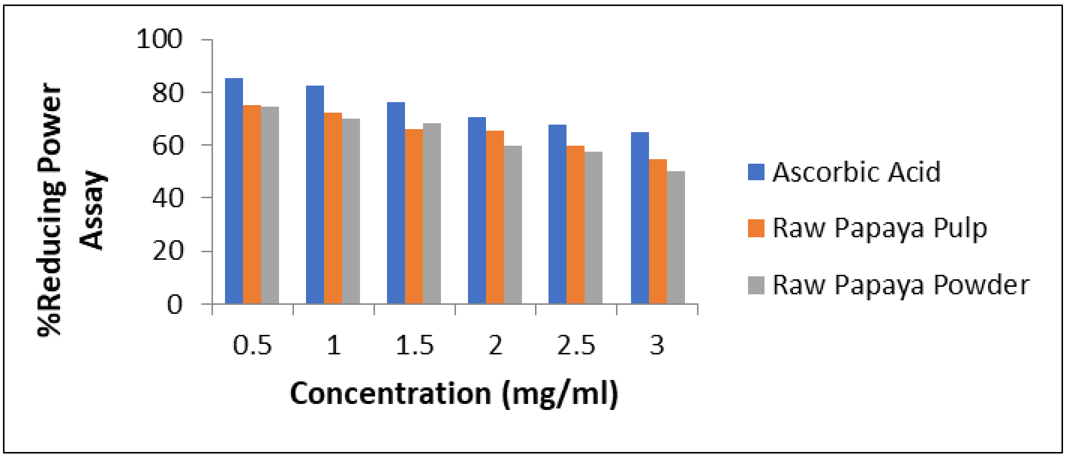

Fig. 4: % inhibition of Ferric Chloride reducing power assay of Raw *C. papaya* Fresh pulp & dried powder

Table 2: Total phenolic content in different samples expressed as *C. papaya* Gallic Acid Equivalent

Sample of *Carica papaya*	Absorbance	TPC as GAE (ppm/20mg sample)	TPC as GAE in (mg/100g sample)
Fresh pulp of ripe	0.90	48	240
Fresh pulp of raw	0.79	39	195
Dried powder of ripe	0.69	35	185
Dried powder of raw	0.52	28	140

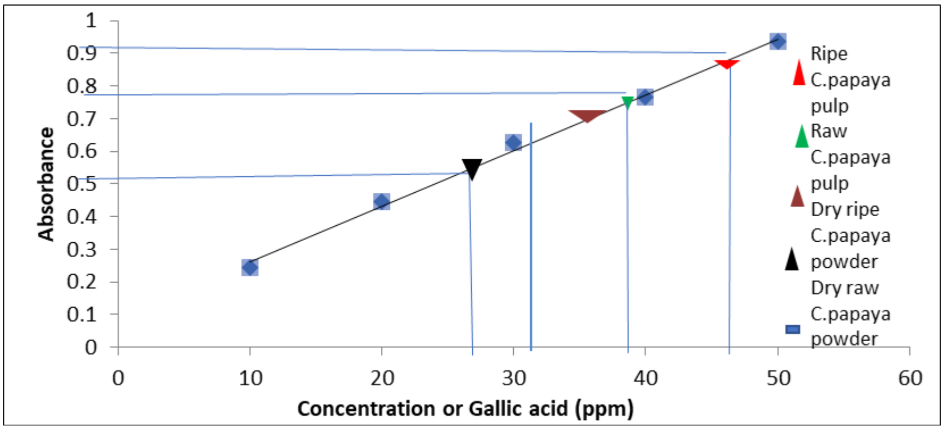

Fig. 5: Calibration plot of Gallic Acid & Gallic Acid Equivalent in various samples

DISCUSSION

The fresh pulp & powder of ripe *C. papaya* shows a very good antioxidant activity (DPPH Radical Scavenging Activity, 80.2%, and 76.4% respectively) which is comparable to the standard drugs such as Butylated Hydroxy Anisole (BHA) (DPPH Radical Activity of Scavenging, 87.2%) and Ascorbic acid. Hence it can be effectively used as an organic substitute for chemical compounds (BHA, BHT) which have a lot of harmful side effects. Scientific studies have supported the pharmacological effects of *C. papaya* mentioned in Ayurvedic texts, and the results show that one of the plant's primary constituents exhibits potent antioxidant, diuretic, antihyperglycemic, anticancer, analgesic, and antidepressant activity.

Phytochemical studies have also shown the presence of phenolic compounds. It is a well-known fact that phenolic compounds are very good antioxidants. Hence, most probably it is these phenolic compounds which are responsible for antioxidant activity (18-19). Thus, total phenolic content (TPC) was evaluated by Folin-Ciocaltaeu's method (20). It was observed that the ripe fruit pulp showed a good value of polyphenols viz. 48 mg Gallic acid Equivalent (GAE)/20 g of the sample.

Another promising fact is that the Antioxidant activity of the dried ripe fruit decreased by only 1.2% and TPC decreased in the ripe fruit from fresh to dried powder by about 23%. Hence the dried powder could be effectively used for preparing antioxidant formulations.

CONCLUSION

The fresh pulp and powder of ripe *C. papaya* shows wide antioxidant activity (DPPH Radical Scavenging Activity. Hence it can be effectively used as an organic substitute for chemical compounds (BHA, BHT) which have a lot of harmful side effects. Moreover, Phytochemical studies of *C. papaya* have shown the presence of Phenolic compounds which are responsible for its potent antioxidant activity. Additional study on the therapeutic efficacy and active principles of *C. papaya* are needed for the purpose to identify the hidden biological element and their clinical use.

ACKNOWLEDGEMENT

Authors are thankful to management DIBNS for their support to carry out this study.

CONFLICT Of INTEREST

Authors have no conflicts of interest of any kind.

REFERENCES

1. Shashni, B., Nagasaki, Y. Nitroxide radical-containing nanoparticles attenuate tumorigenic potential of triple negative breast cancer. Biomaterials. 2018; 178: 48-62.
2. Wu, C.C., Hung, C.N., Shin, Y.C., Wang, C.J, Huang, H.P. *Myrciaria cauliflora* extracts attenuate diabetic nephropathy involving the Ras signaling pathway in streptozotocin/nicotinamide mice on a high fat diet. Journal of food and drug analysis. 2016; 1; 24(1):136-146.
3. Pandey, V.P., Singh, S., Jaiswal, N., Awasthi, M., Pandey, B., Dwivedi, U.N., *et al.*, Papaya fruit ripening: ROS metabolism, gene cloning, characterization, and molecular docking of peroxidase. Journal of Molecular Catalysis B: Enzymatic. 2013;30;98-105.
4. Kamemura, N. Butylated hydroxytoluene, a food additive, modulates membrane potential and increases the susceptibility of rat thymocytes to oxidative stress. Computational Toxicology. 2018; 1;6:32-38.
5. Kumar, N.S., Devi, P.S. The surprising health benefits of papaya seeds: A. J. Pharmacogn. Phytochem. 2017;6(1):424-429.
6. Anitha, M., Selvy, P.T, Palanisamy, V. Automated detection of white matter lesions in MRI brain images using spatio-

fuzzy and spatio-possibilistic clustering models. Computer Science and Engineering. 2012 ; 1;2(2):1.

7. Sripanidkulchai, B., Wongpanich, V., Laupattarakasem, P., Suwansaksri, J., Jirakul Somchok, D. Diuretic effects of selected Thai indigenous medicinal plants in rats. Journal of Ethnopharmacology. 2001 ; 1;75(2-3):185-190.
8. Venkateshwarlu, E., Dileep, P., Sandhya, P. Evaluation of antidiabetic activity of *Carica papaya* seeds on streptozotocin-induced type-II diabetic rats. Journal of Advanced Scientific Research. 2013 ;10;4(02):38-41.
9. Akhtar, P., Yaakob, Z., Ahmed, Y., Shahinuzzaman, M., Hyder, M.K. Total phenolic contents and free radical scavenging activity of different parts of Jatropha species. Industrial Crops and Products. 2018 ; 1;30:365-370.
10. Lokapur V., Jayakar V., Shantaram M. Phytochemical investigation, chemical composition and in vitro antioxidant activities of various crude extracts of Holigarna ferrugenia Marchand. Medicinal Plants-International Journal of Phytomedicines and Related Industries. 2022; 14(1): 72-83.
11. Lokapur V., Jayakar V., Shantaram M. Preliminary phytochemical screening, physicochemical analysis and in-vitro antioxidant activity of selected Holigarna species-Endemic plant species of Western Ghats. Biomedicine. 2020;40(4):460-466.
12. Jayakar V., Lokapur V., Shantaram M. In-vitro antioxidant and selective cytotoxicity of Garcinia cambogia and Garcinia indica leaf extracts on human kidney cancer cell line. International Journal of Research in Pharmaceutical Sciences. 2021 Jul 5;12(3):1718-1728.
13. Lokapur V., Jayakar V., Divakar M.S., Chalannavar R.K., Lasrado L., Shantaram M. ZnO nanoparticles with spectroscopically controlled morphology, bioinspired from Holigarna grahamii (Wight) Kurz and delving its antioxidant and anticancer potential on A498 cell line. Materials Today Communications. 2022 Jun 1; 31: 103338.
14. Jayakar V., Lokapur V., Nityasree B.R., Chalannavar R.K., Lasrado L.D., Shantaram M. Optimization and green synthesis of zinc oxide nanoparticle using Garcinia cambogia leaf and evaluation of their antioxidant and anticancer property in kidney cancer (A498) cell lines. Biomedicine. 2021 Jul 7;41(2):206-222.
15. Jayakar V., Lokapur V., Shantaram M. Identification of the volatile bioactive compounds by GC-MS analysis from the leaf extracts of Garcinia cambogia and Garcinia indica. Medicinal Plants-International Journal of Phytomedicines and Related Industries. 2020;12(4):580-590.
16. Jose M., Varghese V. I., Jayakar V., Lokapur V., Srinivasa K., Shantaram M. Evaluation of antioxidant activities and free radical scavenging properties in mango leaves, husks of areca and coconut. Research Journal of Pharmaceutical Biological and Chemical Sciences. 2018 Jul 1;9(4):1607-1619.
17. Dadoosh, R.M. Evaluation of Phytochemical, Total phenolic and antioxidant activity of *Carica papaya* seed for its use in biosynthesis of gold nanoparticles. Egyptian Journal of Chemistry. 2021; 1;64(8):1-2.
18. Patil, R., Anwar, C., Govind, Vishwas, V., Bapat, R., Jadhav, J.S., *et al.*, Optimization studies for extraction of antioxidants from Mucuna sanjappae seeds: A promising natural drug for oxidative stress management. Biomedicine. 2023; 43(1): 403-412.
19. Vinayaka, K.S., Raghavendra L.S., Prashith, K. Preliminary phytochemical analysis and in vitro antioxidant activity of *Glochidion ellipticum* Wight (Phyllanthaceae). Biomedicine. 2022; 42(1): 148-153.
20. Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent in Methods in Enzymology. 1999; 299: 152-178.