

# Study on Extraction and Quantification of Flavonoids from Phalsa *Grewia Asiatica*

**Jagadesh Prabhu Mahendran\***

Assistant Professor, Department of Management, Dhanalakshmi Srinivasan College of Arts and  
Science for Women, Perambalur, Tamil Nadu

[research@dcollege.ac.in](mailto:research@dcollege.ac.in)

---

## **ABSTRACT**

*Freshly harvested fruits of the tall and dwarf cultivar of the variety Sharbati were evaluated at three different storage temperatures (-5 to -6 0 C, 4 to 6 0 C and 40 to 45 0 C) with subsequent peel and seed drying or freeze drying to determine the appropriate cultivar, its storage life and optimum flavonoid retention in dried pulp. FTIR studies and optimization of process conditions involving extraction techniques (SAE, MAE and UAE), extraction solvents (ethanol, methanol, petroleum ether and water), extraction time and temperature were carried out on the selected cultivar using RSM. In the case of SAE, the time ranged from 1 to 4 h, 15 to 60 min (UAE) and 3 to 12 min for MAE, while the temperature varied from 500C to 1100C for MAE and from 350C to 80C for SAE and UAE. For ethanol, methanol and petroleum ether, the concentration of solvent ranged from 55 to 100 percent when water was used in its pure state. The finding shows that for better preservation of flavonoids and other biochemical constituents for a longer period of time, the dwarf cultivar, deep freeze storage and freeze drying condition were considered optimal. Constituents for longer periods of time. In the case of SAE, optimized conditions for optimized temperature, time and temperature conditions, time and solvent concentration were 64.090C, 2.72 h, 77.72 percent (methanol), for maximum total flavonoid content (TFC) and 65.45 0C, 2.57 h, 77.27 percent (ethanol), for extraction yield, respectively. For the UAE, we obtained maximum TFC quantity and extraction yield at 64.54 0C, 37.72 min, 70.00 percent (methanol) while for MAE, at 76.060C, 9.81 min. 75.00 percent (ethanol). In the case of SAE and UAE, the highest concentrations of TFC were obtained using methanol, while ethanol was found to be the strongest MAE extraction solvent.*

**Keywords** – Quantification, Phalsa

## **INTRODUCTION**

The world scenario shows that India is a leading fruit growing region, followed by China, with its wide variability in soil, environment and physiographic conditions (APEDA, 2015-16). Fruit position The role of fruits in our diet and their potential effect on the improvement of human health in our diet and their potential impact on the improvement of human health have been well known since the beginning of human civilization.

Flavonoids are a category of polyphens that are significant secondary plant metabolites with tremendous medicinal properties, including anti-inflammatory, anti-carcinogenic, antioxidant, anti-mutagenic, etc. India has made significant progress in the production of lesser known fruits such as aonla, ber, bael, sa pota, karonda, mulberry, phalsa, pomegranate, etc. and there has been a steady increase in the area and production of various underused fruits as a result of the growth of appropriate varied relations and improved production technology, as reported by Hasan and Suresh (2008).



**Fig. 1.1: Image of phalsa plant and fruits**

Recently, underutilised small fruit crops have attracted the attention of researchers, farmers and consumers to extend their production area and to provide society with nutritional and economic protection. These crops are easier to cultivate, hardy in nature, even grown under adverse soil and climatic conditions, which have many medicinal uses. The exploitation of under-used indigenous horticultural crops, such as phalsa, can therefore be a solution to the social problems of health, nutrition, hunger and unemployment, thus ensuring our country's food security. Phalsa (*Grewia asiatica* L.), one of the most common small fruit crops of Indian origin (Kacha et al., 2014), belongs to the Tiliaceae family, produces delicious fruits of edible quality, and is capable of withstanding drought and rising and adverse climatic conditions (Debnath et al., 2011). This small fruit crop (Fig. 1.1) is grown in India on a very small scale and is well known for its medicinal properties (Singh et al., 2015). In honour of Nehemiah Grew, one of the founders of plant physiology, the name *Grewia* was given, while *Asiatica* indicates the origin of Asia.

Abid et al. (2012) listed these fruits as non-climacteric fruits, and for marketing, well-ripened fruits are harvested. Some big phalsa-producing countries are India, South Africa, Pakistan, Southeast Asia and the USA (Sinha et al (2015)). It is grown commercially in Punjab, Haryana, Rajasthan, Uttar Pradesh, Madhya Pradesh and is also cultivated in Gujarat, Bihar, Tami Nadu, Maharashtra and West Bengal on a small scale (Kumar et al., 2014). The national total area and production data is not available as an underused minor fruit crop, as it is cultivated on a very small scale in some of the states in our country, while in Punjab, the area under phalsa cultivation is stated to be 30 hectares with annual production of approximately 196 tones (Singh et al., 2015) . Due to its incredible nutritional and medicinal properties, early bearing habits and greater capacity to withstand changing agro-climate conditions, Phalsa occupies an important position among Indian indigenous fruits. Therefore, it should be grown and used on a wide scale in the future (Singh et al., 2012). Highly delicious, sour to sweet berries are ripe fruits with a desired nice flavour and cooling effect, containing 50-60% juice, 10-11% sugar and 2.0-2.5% acid with a good amount of vitamin A as well as C and a reasonable amount of phosphorus and

iron (Kacha et al., 2012 ). The existence of delphinidin - 3-glucoside and cyanidin -3-glucoside, as found in Khurdiya, may presumably be due to the attractive fruit color (1979).

## **OBJECTIVES**

1. To assess physico- chemical composition of composition of fresh and stored and stored phalsa.
2. To estimate flavonoid stability in processed products of phalsa

## **PHALSA PHYTOCHEMICALS**

Now-a-days of photochemistry, because of their enormous ability to treat different health problems, biologically active compounds are becoming essential in the nutritional and medicinal fields. In addition, crude phytochemicals serve a large pool of various therapeutic potential properties. Among fruits, as indicated in different qualitative and quantitative studies, berries such as phalsa serve as excellent sources of several bioactive compounds. While several leaves, stem and bark studies are on record, very little work on bioactive constituents and other functional properties of fruit pulp has been recorded. Almost all previous current photochemical studies on phalsa are focused on qualitative screening and there are a few studies available for photochemical quantitative study. In addition to anthocyanins, ethanol fruit extract also contains flavonoids, phenolic acids and glycosides (in large quantities), alkaloids, steroids and saponins (in moderate quantities), tannins (in small quantities), resins and terpenoids (absent) as described by K. Phalsa is a dark coloured fruit and this dark purple colour of phalsa is due to the presence of anthocyanins (a water soluble pigment).

Sofia et al. (2014) studied two forms of mulberry (white and black) fruits from local areas for different chemical components such as fat content, total antioxidant potential and content of polyphenols. The results of their analysis showed that among both types of mulberry fruits, black mulberry fruit had the highest total phenolic content. Therefore, it can be considered that dark coloured fruits are healthy phenolic sources. Using paper chromatography, Khurdiya and Anand (1981) acquired two anthocyanin pigments, i.e. delphinidin - 3 - glycoside and cyanidin - 3 - glucoside in phalsa fruit. The quantitative photochemical composition of seven tropical fruit residues left after juice extraction was analysed by Gupta et al. (2014) and found that phalsa contained 12.42 mg/g flavonoid (as catechin equivalent), 1.56 g/100g alkaloid, 1.05 g/100g saponin, 0.52 g/100g tannins, which are expected to have antioxidant activity and are responsible for various biological functions. Gupta asserted the existence in phalsa fruit of carbohydrates, vitamin C flavonoids, phenols, saponins, alkaloids, glycosides, hormones, acids, mucilage and tannin compounds in separate studies. The photochemical profile of crude metabolic extracts of this fruit and some other native plants was studied by Srivastava et al (2012). The results of their analysis showed that total flavonoids (4,608 QE mg/g), total phenols (144.11 mg GAE/g) and total anthocyanin (4.88 mg/kg) were present in the fruit. In addition, moderate amounts of total phenols, i.e. 55 -87 mg/ 100g, were also obtained in this fruit by Kaur and Kapoor (2005). Via this fruit. Khanal et al. . Two species from genus species from the Grewia genus i.e. analysed (2016) analysed G. Asiatic and Grewia optima, for their photochemical and biological properties, of the Tiliaceae family.

## **PHALSA: HEALTH BENEFITS**

The less used phalsa plant has numerous health advantages. In the prevention and cure of various diseases, this fruit plays a significant role, which is shown by a large number of extensive studies. Phalsa fruit functions as a phytomedicine and is considered an alternative source of petroleum, hydrocarbons and photochemical products. It serves as a blood purifier and it is understood that whole plants (pulp, seeds, leaves, bark and flowers) have various beneficial health benefits that are well known from ancient times. These fruits are known for the treatment of various diseases such as fever, throat problems, heart, respiratory and blood disorders, as well as anti-cancer, antioxidant, anti-rheumatic, anti-diabetic, hypoglycemic, antipyretic, anti-inflammatory, radio-defensive anti-malarial, anti-ulcer hepatoprotective and anti-hyperglycemic activities. Ripe fruits have astringent, stomachic and aphrodisiac properties, whereas unripe fruits are known to relieve inflammation and to suppress vata, kapha and biliousness. It is stated that these fruits were historically used during summers as cooling agents, anti-inflammatory agents and as a refreshing drink, as well as for the healing of certain urological diseases. Ethanol extract from the fruit showed hypoglycemic activity *in vivo*.

The phalsa fruit was recommended for the treatment of anoxia, diarrhoea, worm and weakness. It is also useful for quenching thirst and calming burning sensations due to heat. Large data on antioxidant, antifungal, antimicrobial, antiviral, anticancer, radio-defence, anti-diabetic, chemopreventive, antiemetic, anti-hyperglycemic, anti-platelet, analgesic, antipyretic and immunomodulatory effects of these fruits are used throughout the literature. Gupta et al. (2006) reported that phalsa fruit also helped in the ejection of a dead foetus, and because of the presence of phytochemicals, minerals and vitamins, this herb was well reputed for its various therapeutic uses. Each part of this plant has been widely used in polyherbal Ayurvedic preparations in the traditional Indian medicine system for the treatment of various ailments as shown in the extract of this fruit.

As mentioned by Singh et al., it also acts as an aphrodisiac agent (2012). The crude ethanol extracts of *G. asiatica* fruit stem bark and leaves for anti-hyperglycemic effects were analyzed and concluded that these extracts demonstrated important anti-hyperglycemic activity as alloxan-induced diabetic rabbits decreased serum glucose levels.

Khanal et al. (2016) found that the extract of n-hexane from *G. asiatica* has shown an anti-inflammatory effect. Antipyretic and analgesic behaviors were examined in Swiss albino mice. They concluded that aqueous extract has strong antipyretic and analgesic activities (dose 200 - 300 mg/kg). They also stated that juice was used for rheumatism and leaves have anti-tubercular action in heart disease, alcoholism, gynaecological disorders and roots. It's Gupta et al. An *in vitro* research on cytotoxic activity against cervical cancer cells, breast cancer cells and hepatocellular carcinoma cells was performed (2014), and seven fruit residues were also studied for alpha amylase inhibition. The extract of *G. asiatica* was suggested by the results of their analysis. *asiaticapomace* was significantly effective against breast cancer cell among all the tested cell lines as well as this extract possessed moderate anti-diabetic activity. Marya et al. (2011) also evaluated the anticancer activity of aqueous extracts of leaves and fruits of this fruit using MTT assay, and results showed that these sections were successful against liver and breast cancer. In order to find the effect of phalsa fruit on glycemic index (GI) and phagocytosis in non-diabetic

humans, Mesaik et. al. (2013) performed an experiment and concluded that fruit had a low value of 5.34 with mild hypoglycemic operation.

## **CONCLUSIONS**

During 2013-2016, the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar (Haryana), performed the extraction and quantification of flavonoids from phalsa (*Grewia asiatica*). Fresh phalsa fruits of the Sharbati variety (tall and dwarf cultivars) were obtained from Central Fruit Farm, Hisar in a properly matured (dark purple) stage during the month of June. On the basis of physical parameters (shape, size, colour, maturity status and visual appearance) observed visually and by determining the total flavonoid material, the first experiment was conducted for the selection of the phalsa cultivar. In addition, attempts were made to research the shelf life of both indoor cultivars (40-45 °C), refrigerated (4-6 °C) and freezing temperatures (-5 to -6 °C). Using tray drier (50±2 °C) and freeze drier (lyophilizer) (-75 °C) for the preparation of dry powder, pulp and seeds of fresh fruit were dried. In the lyophilized dwarf phalsa cultivar, maximum amounts of flavonoids were found. It was therefore selected for further continuation of this research. Physical and chemical composition (moisture content, ash content, protein content, TSS, sugar content (reduction and non-reduction of sugar, tannins, titratable acidity, vitamin C content, total phenol and total anthocyanin content, minerals and heavy metals) of selected dwarf cultivars (fresh fruit, lyophilized pulp pulp); Using FT - IR, functional groups of lyophilized pulp and seed powder have been established. Four solvents (ethanol, methanol, petroleum ether and water) and three extraction techniques (Soxhlet [Soxhlet - assisted extraction (SAE), ultrasound assisted extraction (SAE), ultrasound - assisted extraction (UAE) and microwave assisted extraction (UAE) and microwave - assisted extraction (MAE) were used to extract lyophilized pulp powder (LPP) flavonoids.

## **RESEARCH GAP AND SIGNIFICANCE OF THE STUDY**

Phalsa fruit, despite its significant nutritional and photochemical characteristics, has not yet gained much popularity among fruit growers and processed fruit industries in India. This fruit continues to grow unattended; no systematic approach is pursued, as information and routine cultivation of this fruit is still scarce. Natural antioxidants are the main driving force in the modern period for researchers, food technologists, nutritionists, consumers and the pharmaceutical industry. Due to its perishable existence and seasonal availability, the utilization of phalsa fruit for the extraction of bioactive compounds is still unexplored. Very limited scientific data on photochemical composition, flavonoid extraction and quantification are available, along with the possible use of flavonoid extracts to enhance nutraceutical and therapeutic values in processed food items. In recent times, fortification of processed foods with flavonoid extract has been encouraged to ensure the minimum daily requirement for immune system enhancement. Phalsa fruit may be an option for the extraction of bioactive compounds as food supplements, if it meets this purpose. Keeping in mind the importance of this crop, it is important to investigate the above aspects for enhanced awareness, improved utilisation and increased acceptability between producers and consumers. Flavonoid extraction and quantification are therefore very important for the commercial use of this crop. However, due to the inherent diversity of flavonoids, it is difficult for analytical chemists and biochemists to

measure rapidly and systematically, and accessible literature on these aspects of research is very scarce.

## REFERENCES

- [1] Abdelwahed, N. A. M.; Ahmed, E. F.; El- Gammal, E. W. and Usama, W. H. (2013). Application of statistical design for the optimization of dextranase production by a novel fungus isolated from Red Sea sponge. *3 Biotech* 4: pp. 533-544.
- [2] Abid, M.; Muzami I, S.; Kirmani, S. N.; Khan, I. and Hassan, A. (2012). Effect of different levels of nitrogen and severity of pruning on growth, yield and quality of phalsa (*Grewia subinaequalis* L.). *African Journal of Agricultural Research* 7(35): pp. 4905-4910.
- [3] Abidin, L.; Mujeeb, M.; Mir, S. R.; Khan, S. A. and Ahmad, A. (2014). Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of *Vitex negundo* Linn. by HPLC. *Asian Pacific Journal of Tropical Medicine* 7(Suppl 1): pp. S289-S293.
- [4] A biodun, O. A. and Akinoso, R. (2014). Physico - chemical properties of serendipity berry (*Dioscoreophyllum cumminsii*) fruit. *Journal of Applied Science and Environmental Management* 18(2): pp. 218-221.
- [5] Adeola, A. A. and Aworh, O. C. (2014). Effects of sodium benzoate on storage stability of previously improved beverage from tamarind (*Tamarindus indica* L.). *Food Science & Nutrition* 2(1): pp. 17-27. DOI : 10.1002/fsn3.78.
- [6] Agrawal, S. and Misra, K. (1979). Phytochemical study of the fruit pulp of *Grewia asiatica* Linn. *Journal of the Indian Chemical Society* 56(6): pp. 649.
- [7] Bimakr , M.; Rahmana, R. A.; Taipa, F. S.; Ganjloob, A.; Salleh, L. M.; Selamat, J.; Hamid, A. and Zaidul, I.S.M. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food and Bioproducts Processing* 8 (9): pp. 67-72.
- [8] Franco, M. C. C.; Chimal, C. R.; Hernández, M. G. H.; Colín, C. A. N.; Martínez, M. A. H. and Maldonado, S. H. G. (2014). Physicochemical, nutritional and health - related component characterization of the underutilized Mexican serviceberry fruit (*Malacomeles denticulata* (Kunth) G. N. Jones). *Fruits* 69: pp. 47-60.
- [9] Gupta, P.; Bhatnagar, I.; Kim, S.; Verma, A. K. and Sharma, A. (2014). In- vitro cancer cell cytotoxicity and alpha amylase inhibition effect of seven tropical fruit residues. *Asian Pacific Journal of Tropical Biomedicine* 4(2): pp. S665-S671.
- [10] Hassimotto, N. M. A.; Mota, R. V. D.; Cordenunsi, B. R. and Lajolo, F. M. (2008). Physico - chemical characterization and bioactive compounds of blackberry fruits (*Rubus* sp.) grown in Brazil. *Ciencia e Tecnologia de Alimentos* 28(3): pp. 702-708.

- [11] Haq, M. Z. U.; Ahmad, S.; Imran, I. ; Ercisli, S. and Moga, M. (2015). Compositional study and antioxidant capacity of *Grewia asiatica* L. seeds grown in Pakistan. *Comptes rendus de l Acad emie bulgare des Sciences* 68(2): pp. 191-200.
- [12] Joshi, K. C.; Prakash, L. and Shah, R. (1974). C hemical investigation of the bark and heartwood of heartwood of *Grewia asiatica* *Grewia asiatica*. *Journal of the Indian Chemical Society* 51(9): pp. 830.
- [13] Kacha , H.L.; Viradia, R. R.; Leua, H. N.; Jat, G. and Tank, A. K. (2012). Effect of NAA, GA3 and ethrel on yield and quality of phalsa (*Grewia asiatica* L.) under South - Saurashtra condition. *The Asian Journal of Horticulture* 7(2): pp. 242-245. <https://shodhganga.inflibnet.ac.in/handle/10603/278440>