Journal of Global Biosciences
Peer Reviewed, Refereed, Open-Access Journal
ISSN 2320-1355
Volume 10, Number 11, 2021, pp. 9117-9127
Website: www.mutagens.co.in
URL: www.mutagens.co.in/jgb/vol.10/11/101106.pdf

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Research Paper

ESTIMATION OF PHENOLIC COMPOUNDS AND SOME PIGMENTS FROM *Hibiscus sabdariffa* L.

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Abstract

The present study was undertaken to find out phenolic compound content and pigments of flowers of *Hibiscus sabdariffa* L**.** Collection of plant material were done from Melghat forest region, Dist.- Amravati, Maharashtra. *Hibiscus sabdariffa* L**.** is annual herbaceous shrub, cultivated for its flowers although leaves and seeds have also been used in traditional medicine . The calyces of the plant are used as a refrigerant in the form of tea, to make jellies and jams. Plants were identified with the help of the standard floras.1gm of were calyces used for determination of Phenolic compounds & some pigments. Phenolic compound act as antioxidant. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. Estimation of total phenol, quinones, flavonols and tannins were done and estimation of some pigments Anthocyanin, Leuco-anthocyanins and total carotenoids were done.

Key words: *Hibiscus sabdariffa* L**. ,** Phenolic compounds, pigments, Spectrophotometer.

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative Health care since time immemorial. It has been reported that phytochemicals, nonnutritive chemicals present in fruits and herbs may protect human from a host of diseases for their biological activities (Argal and Pathak, 2006). Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases.

A variety of plant secondary metabolites have been reported to act as antioxidants and amongst them phenolic compounds from a major group. There are several reports on the contribution of phenolic compounds to the antioxidant potential

of different plant species (Cai *et al.,* 2004). flavonoids are naturally occurring phenolic compound which largely include anthoxanthins (flavones, flavonols, flavanones, flavanols, chalcones and isoflavones), anthocyanins, leucoxanthins and flavonoidal alkaloid (Houghton, 2002).

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state.

Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions and such chain reaction when occurs in a cell, results into the damage or death to the cell.

Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A and E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidant cause oxidative stress and may damage or kill the cells. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart diseases and even altitude sickness.

Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack our cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins and enzymes present in the body. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body by use of oxygen in respiration and some cell-mediated immune functions. They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pestisides etc. (Li and trush, 1994).

The major antioxidant capacities of plants such as fruits and vegetetables are vitamins C and E and phenolic compounds, especially flavonoids. Phenolic compounds posses different biological activities , but most important are antioxidant activites . phenolics are able to scavenge reactive oxygen species (ROS) due to their electron donating properties. The antioxidant effectiveness in food depends on not only the number and location of hydroxyl group but also on factors such as physical location , interaction with other food components, and environmental conditions .In many studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Re *et al*.,1999 and Velioglu *et al*.,1998).

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retarding the lipid oxidative rancidity in foods .the potential of plant products as antioxidants against various diseases induced by free radicals.the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids and phenolic acids.

MATERIALS AND METHODS:

Plant material

***Hibiscus sabdariffa* L.**

A genus belonging to Malvaceae family, erect glabrous shrubs. Stem tinged with red. Leaves entire 3-5 lobed, 5-10cm long, cuneate at base; lobes lanceolate or oblong, 2.5-9.5× 0.6-2.5 cm, green or red, serrateth, glandular on midrib beneath; petioles 3-6 cm long, reddish purple; stipules linear, 1-1.5 cm long. Flowers 2-5cm across, solitary, axillary. Pedicels short, stout, jointed near base. Bracteoles 10, linear lanceolate, shorter than the calyx and adnate to its base, red purple. Calyx 1-1.5 cm long, much enlarged in fruit, divided up to the middle, lobes fleshy, lanceolate. Corolla ovoid, beaked, enclosed in fleshy, persistant calyx and bracteoles. Seeds reniform, black or dark brown, covered with minute, stellate hairs.

Flowers and fruits : October to January

Uses

The plant has been used as antiseptic, astringent, cholagogue, aphrodisiac,demulcent, emollient, purgative, stomachic, antinociceptive, antiflammatory, sedative,tonic and also used for high blood pressure, liver diseases, fever, ulcers, abcesses andanemia.(Mahadevan *et al.,*2009). Cytotoxicicity and antibacterial, Antioxidant. (Bako *et al.,*2009). The extensive survey, identification and collection of plant from Melghat region was carried out. Plant identification was carried out with the help of floras (Cook,1957; Dhore,1986;1998; Naik , 1998).

Preparation of plant material

Fresh flowers were collected and the dried in sunlight. After them powdered with mechanical grinder and stored in airtight container. samples were powdered separately. 1gm each of samples were taken for estimation of phenolic compounds and pigments grm/ µgm.

Methods:

Estimation of Phenolics such as total phenol, Quinones, Flavonols and Tannins were done according to the methods prescribed by Thimmaiah (1999), which are given below.

Estimation of Total Phenols

1gm of sample was grind with the help of mortar Pestle with 10 ml of 80% ethanol. The homogenate was centrifuged for 20 mininutes at 10,ooo rpm. Supernatant was collected. Supernatant was Evaporate to dryness.

Then after dryness residue was taken and make up the volume with 5ml distilled water. 1 ml aliquate was Pippette out in test tube , and volume make up 3 ml with distilled water . To it 0.5 ml of Folin- Ciocalteu reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added into each tube.

Mixed thoroughly and tubes was kept in boiling water for 1 minute, then allowed to Cool and absorbance at 650 nm was measured against reagent blank.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999)

Estimation of Quinones

1gm sample was grind with the help of mortar and pestle with using chilled phosphate buffer (5ml for each gm of tissue). The supernatant was collected by Centrifugation for 30 minutes this was used as enzyme extract. 3ml of buffer, 3ml of standard catechol and 1.5 ml of enzyme extract was pipette in a test tube. It was shaken gently and incubated in water bath. 4ml of TCA (Trichloro acetic acid) reagent (without ascorbic acid) to one and 4ml of TCA reagent (with ascorbic acid) was added. Precipitate was filtered. Absorbance was measured at 400 nm against a reagent bank lacking only extract.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of working standard catechol. (Thimmaiah S. R. 1999)

Estimation of Flavonols :-

1gm sample was grind with the help of mortar and pestle with 10ml of ethanol and the supernatant was collected by centrifugation for 20 minutes.The

supernatant was evaporated to dryness; then the residue was dissolved in 5 ml distilled water. 1ml of extract was pipette out into 25ml conical flask and 1 ml of distilled water was added.

Then 4ml of vanillin reagent was added from a burette rapidly within 10-15 sec to flask A and 4ml of 70% H_2SO_4 to flask B.

A blank was prepared in flask C containing 4 ml of vanillin reagent and 2ml of distill water. Shaken the both flasks A and B in a water bath at the temperature below 35^0C. Keeping the flasks at room temperature for exactly 15min. Absorbance was measured flask A, B and C at 500 nm against 47%. H_2SO_4 (flask D).

The absorbance of the flasks B and C from that of A.The flavonol content was calculated using a standard curve prepared from phlorogucinol or kaempferol (100 µg/ml). (Thimmaiah S. R. 1999)

Estimation of Tannins :-

Vanillin hydrochloride method was used.

1 gm of sample was mixed in 10ml methanol after 20-28 hrs. centrifuged and supernatant was collected .pipette out 1ml supernatant into test tube and quickly 5ml of vanillin hydrochloride reagent was added and mixed. After 20 min absorbance was read at 500nm. A reagent blank was prepared with vanillin hydrochloride reagent alone. A catechin standard graph was prepared from working standard (100µg/ml) of catechin and amount of tannins was calculated. (Thimmaiah S. R. 1999)

Estimation of Anthocyanins

1 gm of sample was grind with the help of mortar and pestle by using absolute alcohol. Centrifuge and the extract was collected. 1ml of alcohol extract was pipetted in test tube and 3ml of HCL in aqueous methanol was added. 1ml of anthocyanin reagent was add in the samples. The Blank was prepared in the same manner by adding 1 ml of methanol - HCL instead of anthocyanin reagent. After 15min of incubation in dark, Absorbance was measured at 525 nm After 15min of incubation in dark, Absorbance was measured at 525 nm against the bank laking only extract. (Thimmaiah,S. R. 1999)

Estimation of Leuco-Anthocyanins

1gm of sample was grinded with the help of mortar and pestle by using 10 ml methanol. Centrifuge and supernatant was collected. Pipette out 1ml of extract in test tube and volume was reduce to 0.5 ml on a hot water bath.0.5 ml of distill water and 10ml of anthocyanin reagent was mix thoroughly in reduced extract. The tubes was heated in water bath for 3 min without covering and with covering total for 40 minute. After heating cool under a running tap. Absorbance was measured at 550 nm against a reagent blank laking only extract. (Thimmaiah, S. R. 1999).

Estimation of Total Carotenoids

1 gm of sample was grinded with the help of mortar and pestle by using 10ml of acetone. Extract was filter on Buchner Funnel through whatman no. 42 filter paper. The extraction was repeated until the tissue was free from pigments. Pool the filtrates and partition thrice equal volume of peroxide free ether was using seperatory funnel. For producing two layers during initial ether extraction distilled water was added. Hot water bath was used for evaporation of the combined ether layer which contains carotenoid. The residue was dissolve in 5ml of ethanol. 0.5 ml of 60% aqueous KOH was used for 10 ml of the ethanol extract to saponify.,The mixture was kept in the dark forovernight at room temperature. After overnight equal volume of water and equal volume of diethyl ether was used for partition. Evaporate the combined ether layer as before and the residue was added in minimum volume of ethanol. Absorbance was measured at 450 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1ml) of β-carotene. (Thimmaiah,S. R. 1999). After 15min of incubation in dark, Absorbance was measured at 525 nm against the bank laking only extract. (Thimmaiah,S. R. 1999)

A standard curve is the plot obtained by plotting concentration of a given standard along X- axis and the corresponding absorbance values along Y- axis on a graph sheet resulting a straight line which passes through the origin. It is used to quantify the amount of a given compound present in an unknown sample whose absorbance value is matched against that of standard along Y-axis and a corresponding concentration could be read off along X-axis. . (Thimmaiah S. R. 1999)

RESULT AND DISCUSSION

Table 1: Estimation of Phenolics

Sr. No	Name of plant and part used for estimation	Total Phenol		Flavonol		Quinone		Tannin	
1	*Hibiscus sabdariffa* (calyces)	Absorbance (650nm)	2700µgm/gm	Absorbance (500nm)	0.78µgm/gm	Absorbance (400nm)	50µgm/gm	Absorbance (500nm)	480µgm/gm

Table 2 : Estimation of Pigments

Sr.No.	Name of plant and part used for estimation	**Total Carotenoids** Absorbance at (450nm)	**Anthocyanins** Absorbance at (525nm)	**Leuco-Anthocyanins** Absorbance at (550nm)
1	*Hibiscus sabdariffa* (calyces)	43µgm/gm	29.12µgm/gm	4.65µgm/gm

Highest amount of total phenol in calyces (2,700µgm/gm) and tanin was observed in calyces of *Hibiscus sabdariffa* (480 µgm/gm) and while lowest content of flavonol in calyces (0.78µgm/gm). However there is significant amount of quinone was found (50µgm/gm). In *Hibiscus sabdariffa* calyces the rich amount of (43 µgm/gm) total carotenoids was found.

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. (Rice-Evans *et al.,*1997). The nitric oxide scavenging activity of flavonoids and phenolic compounds are known (Kim H. *et al.,* 2002) .

Phenols are present in food, they may have an impact on health and most are known to have an antioxidant activity. (Demitrios 2006) .

Phenols and polyphenolic compounds such as flavonoids are widely found in plant sources and they have been shown to posse's significant antioxidant activities (Van Acker S. *et al.,* 1996).(Akanaya *et al*., 1997)The presence of Tannins, cyanogenic glycoside andsaponins had reported in calyces of *Hibiscus sabdariffa*. (Chopra *et al*., 1986)recommend *Hibiscus sabdariffa* for lowering blood pressure.

Medicinal plants contain some organic compounds which provide definite physiolgical action on the human body and these bioactive substances include tannins, alkaloids, flavonoids, carbohydrates, herbenoids and steroids.(Edoga *et al.,* 2005; Mann 1978).

Herbs that contain tannins are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery. (Dharmananda, 2003). Flavonoids are one of Major Classes of Phytochemical associated with diverse pharmacological activities which include antioxidant, anticancer and anti ageing properties.(Sharma, 2006). (Pietta, 2000) was studied the dried calyces of *Hibiscus sabdariffa* contain the flavonoids-gossypetin, sabdaretin, hibiscetin and anthocyanins. There are indications that the extract from the red calcyes of possess antioxidant principles (Tseng *et al.,* 1997; Wang *et al*., 2000)

Carotenoids may also have important anti-ageing and anticancer properties such lutein, which protects the tissues of retina, while canthaxanthin has been demonstrated to inhibit cancer cell proliferation (Palozza *et al*., 1998).

Anthocyanins possess a high the rmost ability and contribute towards antioxidative, antiflammatory, cardioprotective and hepatoprotective activities. The anthocyanin extract from dried calacyes of *Hibiscus sabdariffa* protects the rabbit against 2,4 -DNPH Lipperoxiative and cytotoxic effects(ologundudu *et al.,* 2010)

Anthocyanins have shown Angiotensin converting enzyme (ACE) inhibition in vitro Delphinidin 3-0 Sambubiosides and Cyanidin -3-0 Sambubiosides isolated from *Hibiscus sabdariffa* extract (Ojeda *et al.,* 2010). The mechanisms behind the reduction of blood pressure by anthocyanins were reported due to their antioxidant activity (Shindo *et al.,* 2007).

CONCLUSION

This study revels that the calyces of *Hibiscus sabdariffa* L**.** contain rich total phenol, quinone and tannin, flavonoids and pigments which are known to posses good source of antioxidant activity and anti-inflammatory activity. The reported phytochemical studies support its traditional uses and may prove to be useful for clinical evaluation. The use of natural antioxidants has been promoted because of the concerns on the safety against synthetic drugs.

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