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ORIGINAL ARTICLE

# An analyses of flavonoids present in the inflorescence of sunflower

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**Abstract**
Flavonoids are low molecular weight secondary metabolites present ubiquitously in plants. They are known to have diverse metabolic functions, including regulation of growth and development, protection against biotic and abiotic stress, male fertility and pollen–stigma interaction. Present investigations deal with the importance of flavonoids in reproductive biology of sunflower (*Helianthus annuus* L. 1753), a member of family Asteraceae. A relationship among flavonoids, phenols and reactive oxygen species has been established in the reproductive component of sunflower. Flavonoids have been quantified, localized and compared using diphenylboric acid 2-aminoethyl ester fluorescent probe, high-performance liquid chromatography, thin-layer chromatography and spectrophotometry. Present investigations reveal that flavonoids and phenols are present in all the components of inflorescence—stigma, pollen, ray and disc florets. Quercetin is the main flavonoid identified in all the components of the inflorescence. Quercetin and kaempferol have been identified in pollen grains. These investigations further reveal that flavonoids act as antioxidants, have a developmental role in stigma, metabolic role in pollen and also play a signalling role in pollen–stigma interactions.

**Keywords** Phenols · Pollen · Pollen–stigma interaction · Stigma

## 1 Introduction

Sunflower (*Helianthus annuus* L. 1753) is an economically important crop, cultivated worldwide for its edible oilseed usage. It is a native plant of North America, Southern Canada and Mexico and has been domesticated by native North Americans 3000 years ago. Besides its use as cooking oil, the crop is also used for edible seeds, nectar and forage. The sun-like radial inflorescence of sunflower, capitulum, develops in such as manner so as to attract maximum pollinators, artists, scientists, and serves as a social icon, with a focus on research, mainly solar tracking and inflorescence development. Due to the diverse use of sunflower, the biochemical components of sunflower have been of interest for various plant scientists. The biochemical constituents not only affect the growth of plants, but also play a role in the interaction of plants with the environment, as they have the ability to act as chemical messengers.

Flavonoids are a group of naturally occurring secondary metabolites, found universally in all plants and prokaryotes. These are classified into different groups including flavone, flavanone, flavonol, isoflavonoid, anthocyanin and chalcones (Harborne and Williams 1998). Flavonoids are known to have diverse functions in plants including protection against ultraviolet (UV) radiations and phytopathogens, colouration of flowers, male fertility, signalling during nodulation and auxin transport (Ferreyra et al. 2012; Agatia et al. 2012). In plants they are also of evolutionary and taxonomical importance (Havsteen 2002). Recent studies indicate that flavonoids act as strong antioxidants and act as powerful tools to scavenge free radicals, reactive oxygen species (ROS) and reduce oxidative damage of tissue. The exact role of flavonoids and different types of flavonoids in sunflower, however, needs to be deciphered. Increased emphasis and interest in the usage of flavonoid calls for a study undertaking the screening of various types of flavonoids and their biochemical activity that may lead to beneficial effects.

This work deals with the quantitative as well as the qualitative analysis of flavonoids present in the different parts of

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the inflorescence of sunflower—pollen, stigma, ray florets and the disc florets.

## 2 Materials and methods

**Plant material** – Seeds of *Helianthus annuus* L. (1753) var KBSH were obtained from the University of Agricultural Sciences, Bangalore (India). Seeds were washed and imbibed in distilled water for 4 h. Imbibed seeds were grown in the garden of Mutanimal Modi College, Modinagar. Plants were raised to reach the reproductive stage. Flowers were excised on anthesis at stage 5.3, according to Scheiter and Miller (1981), when 30% of the head area was in flowering stage. These were used for subsequent experimental work. Morphologically different stages of disc florets were identified (bud, staminate and pistillate), ray florets and pollen were excised for further experiments.

**Solvent extraction** – The inflorescence of *Helianthus annuus* L. (1753) was collected in the month of March–April, upon attaining maturity. The stigmas of various stages were excised and carefully brushed to remove any pollen grain sticking on the surface of stigma. The pollen, ray florets and disc florets were also collected and dried at room temperature. One gram fw each sample was extracted separately in 80% methanol at 50 °C for 12 h in a soxhlet. The extract was filtered, decolourized and defatted with petroleum ether. The extract was then reduced by rotary vacuum evaporator.

**Quantitative detection of anthocyanins** – Anthocyanins were detected using a method provided by Kochhar and Gujral (2012). Five hundred mg of plant material was mixed with 1 ml of extraction solution (methanol 1% HCl) overnight at 4 °C in dark. 500 µl of water was added, and after 5 min. 1 mL of chloroform was added, and the mixture was centrifuged at 10,000 for 10 min. The supernatant was extracted and the final volume was made up to 3 mL by adding a solution of 60 mL of 1% methanolic HCl and 40 mL of $H_2O$. Spectrophotometric absorbance was taken in 530 nm and 650 nm.Total anthocyanin content µg $g^{-1}$ fw of plant material was calculated as:

$$\text{Anth} = \left(A_{535} - 2.2A_{650}\right)$$

**Total phenol estimation** – Total phenol content was estimated using colorimetric assay given by Karimi et al. (2010), with slight modification. Briefly, 100 µL samples were mixed with 1.5 mL of Folin–Ciocalteu reagent (1:10 v/v). After 5 min. 2 mL of 7.5% sodium carbonate was added and the mixture was incubated for 90 min with intermediate shaking. The blue coloured mixture was measured at 760 nm. The standard curve was prepared using gallic acid, and the total phenolic content was expressed as µg gallic acid equivalent $g^{-1}$.

**Total flavonoid estimation** – Total flavonoids were estimated as free and bound fractions. Phytochemicals can exist in free and bound forms. Bound forms are conjugated to the cell wall. The bound flavonoids are thereby subjected to acid hydrolysis (Sharma and Kumar 2008). Briefly for free flavonoids, the samples were dissolved in diethyl ether and then subjected to chloride test. To 1 mL of extract, 2 mL of distilled water was added. After 5 min. 0.15 mL of 5% NaNO2 was added, and after 5 min. 0.15 mL of AlCl3 was added. Subsequently, 2 mL of 4% NaOH was added leading to a final volume of 5 mL. The absorption was measured spectrophotometrically at 510 nm. The standard curve was prepared using rutin, and the free flavonoid content was expressed as µg/mL rutin content. Bound flavonoid content was also measured as per the same procedure, after acid hydrolysis of the samples.

**Antioxidant assay [1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay]** – The DPPH assay is based on the fact that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. DPPH· accepts hydrogen from an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH· in test samples. DPPH· shows a strong absorption maximum at 517 nm (purple). Its colour turns from purple to yellow, followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed, which therefore measures the antioxidant effect, evaluated by a decrease of absorption at 517 nm.One hundred microlitre of plant extract was added to 3 mL of DPPH (0.1 mM) in methanol. The mixture was then shaken and allowed to stand for 20 min. Absorbance was measured at 517 nm using spectrophotometer. The antioxidant activity was expressed as µg ascorbic acid equivalents per g fresh weight.

**TLC and HPLC analysis for flavonoids** – The solvent system standardized for the separation of flavonoids from the methanol extract of different stages/parts of inflorescence of sunflower consisted of a mixture of toluene, ethyl formate, formic acid in the ratio of 50:40:10. Separated components on the in silica plates were observed using a UV lamp. HPLC analysis for flavonoids was accomplished by Waters Alliance Model, consisting of a quaternary pump, online degasser, autosampler, column heater and variable length detector. Separation was achieved on reversed phase C18 column (Thermoscientific, 5 µm, 4.6×250 mm) at 30 °C with diode array detector set at 190 to 600 nm. The mobile phase consisted of 0.1% formic acid & water (solvent A) and

acetonitrile containing 0.1% formic acid (Solvent B). Initially the mobile phase started as 95% A, after 7 min 88% A, after 12 min 82% A, after 17 min 78% A, after 22 min 75% A, after 27 min, 65% A, after 37 min 47% A, and after 42 min 40% A. For the next fifteen min, the flow rate was constant at 20% A, then 15% A for next 8 min, and then brought back to the starting flow rate of 95% A. Injection volume 20 µL (passed through the microfilter), and elution performed at 1.2 ml/min, detected at 280 nm. The solutions of standards at various concentrations were injected into the HPLC system, and the calibration curves were established for each standard compound. The concentration of each compound was calculated from the peak area according to the calibration curves. The amount of the flavonoid detected was expressed as ppm/ml.

**CLSM and spectrofluorometry** – Flavonoids were localized with DPBA, as described by Hsieh and Huang (2007) and Thompson et al. (2010). Briefly, anthers containing mature pollen were fixed in 0.05% glutaraldehyde and 4% paraformaldehyde in phosphate buffer saline (PBS, 0.14 M NaCl, 2.7 mM KCl, 6.5 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, pH 7.3). Fluorescence was observed following UV excitation of tissue stained with 0.1% DPBA (diphenylboricacid 2-aminoethylester in 0.1 M potassium phosphate buffer pH 6.8,1% NaCl). The tissues were excited at 405 nm and viewed at an emission of 580 nm using diode laser and a pin hole of 600 µm (Leica, USA). For DPBA-stained spectrofluorometric analysis, 100 µM DPBA was added to the tissue extract at 1:1 (v/v) as per Lee et al. (2014), and then the emission was measured using an excitation filter at 485 nm and emission filter at 535 nm (PerkinElmer, USA).

All experiments were done in triplicates with at least three technical replicates in each experiment. Statistical analysis, wherever required, was undertaken by calculating standard deviations ad errors along the mean data from replicates.

## 3 Results

**Inflorescence of sunflower consists of morphologically distinct components** – The inflorescence of sunflower is a capitulum, consisting of numerous florets arranged in a spiral pattern. The outer whorl of the florets is zygomorphic, ligulate and incomplete (ray florets), while the inner florets (disc florets) are actinomorphic, ligulate and complete (Fig. 1a). The ray florets are formed by the fusion of 3–5 elongated petals which are flattened to form a ribbon-like structure with a short corolla tube at one end. The corolla tube has a vestigial ovary with/without vestigial stigma (Fig. 1f). The disc florets have a tubular corolla and an inferior ovary (Fig. 1e). Inside the corolla tube is the anther column with five fused anthers. The style is inside the anther tube which elongates at maturity, exposing the bifid stigma. Nectary is at the base of corolla tube on top of the ovary, at the base of the style. Due to the centripetal maturation pattern of the disc florets, morphologically variant disc florets can be observed in the same inflorescence. Bud stage consists of perianth, corolla, androecium and gynoecium (Fig. 1b). The corolla tube contains the clasped stigma and pollen grains inside the anther lobe. At the staminate stage, the anther lobes are ejected from the tubular corolla, and the pollen grains are soon released as three-celled structure (Fig. 1c). At the pistillate stage, the style elongates through the anther lobe and detaches along its median, exposing the forked stigma (Fig. 1d).

**Stigma contains high amount of phenols** – Phenolic are known to provide resistance against various pathogens and increase in stressful situations. Present investigations reveal that phenol content is highest in the pistillate stage of stigma (Fig. 2). It increases gradually from bud stage 1000 µg $g^{-1}$ to 1500 µg $g^{-1}$ in staminate stage and 2000 µg $g^{-1}$ in the pistillate stage. The phenol content of pollen is similar to the phenol content of stigma in the staminate stage. The phenol content is lowest in the ray florets 650 µg $g^{-1}$ and the disc florets 500 µg $g^{-1}$.

**Anthocyanins are present in all the components of the sunflower inflorescence** – Anthocyanins are water-soluble pigments present in different parts of the plants. Together with flavonoids, they are responsible for the colouration and sweet aroma in plants parts. Present investigations reveal that anthocyanins are present in all the parts of inflorescence. The anthocyanin content, however, is maximum in the pollen (8 µg $g^{-1}$) followed by the disc florets (7 µg $g^{-1}$), the stigma stages and the ray florets (4 µg $g^{-1)}$. Minimal anthocyanin content is present in the bud stage (2 µg $g^{-1}$) and then increases suddenly in the staminate and pistillate stage (6 µg $g^{-1}$).

**Pollen contains maximal content of flavonoids** – The free flavonoid (Fig. 2) content gradually increases in the stigma, reaching a maximum in the pistillate stage (175 µg $g^{-1}$). The bud stage of stigma contains higher free flavonoid content than the staminate stage. The pollen, however, contains the highest amount of free flavonoids 200 µg $g^{-1}$. From among the disc and the ray florets, the ray floret contains higher amount of free flavonoids. All the stages of stigma, pollen, ray and disc florets contain higher proportion of bound flavonoids than the free flavonoids. Pollen contains the highest bound flavonoid content (450 µg $g^{-1}$). Bound flavonoid content increases at the stigma stage from 90 to 300 µg $g^{-1}$. Ray and disc florets also contain higher amounts of bound flavonoid 100 µg $g^{-1}$ and 150 µg $g^{-1}$, respectively. The ratio


**Fig. 1** Sunflower. **a** Capitulum of sunflower bearing ray and disc florets. **b** Disc floret of bud stage. **c** Disc floret of staminate stage. **d** disc floret of pistillate stage. **e** Disc floret bearing tubular corolla. **f** Ray florets

of free flavonoids to bound flavonoids content is highest in the bud stage and almost double in all the other parts of the inflorescence in sunflower.

**Quercetin is the major flavonoid in sunflower** – TLC preparations show the presence of quercetin in all the components of the inflorescence (Fig. 3). Pollen contains many other flavonoids in low quantities, one of them being kaempferol. Quantification of the flavonoids by RP-HPLC reveals that the amount of quercetin is highest in the pistillate stage of stigma (Fig. 4). In stigma, quercetin content is lowest at the bud stage (200 ppm/ml) which increases to 350 ppm/ml in the staminate stage and then suddenly becomes threefold in the pistillate stage (900 ppm/ml). The quercetin content of the pollen (700 ppm/ml) is less than that of the stigma of pistillate stage The quercetin content of the ray florets is similar to that stigma in the bud stage (200 ppm/ml) and lowest in the disc floret (180 ppm/ml). Spectrofluorometrically, flavonoid content is highest in the pistillate stage (0.65 mg $g^{-1}$) and declines at staminate stage (0.5 mg $g^{-1}$) and bud stage (0.3 mg $g^{-1}$). In pollen flavonoid content (0.84 mg $g^{-1}$) is higher than stigma. Among the ray floret and disc floret, flavonoid content is higher in disc floret (0.4 mg $g^{-1}$).

Flavonoids were further examined by CLSM after DPBA staining. Flavonoids were present on the surface of mature pollen. Flavonoids could also be localized in between and within exine.

**Flavonoids and Phenols act as antioxidants** – The antioxidant content is highest in the pistillate stage of stigma. The antioxidant content increases from the bud stage of stigma (70 µg $g^{-1}$ fw), ascorbic acid equivalent to 80 µg $g^{-1}$ fw in staminate stage and 100 µg $g^{-1}$ fw in the pistillate stage. The antioxidant content in pollen was the highest (110 µg $g^{-1}$ fw). The antioxidant content of the disc florets and the ray florets was lowest (65 µg $g^{-1}$ fw) and (60 µg $g^{-1}$ fw), respectively.

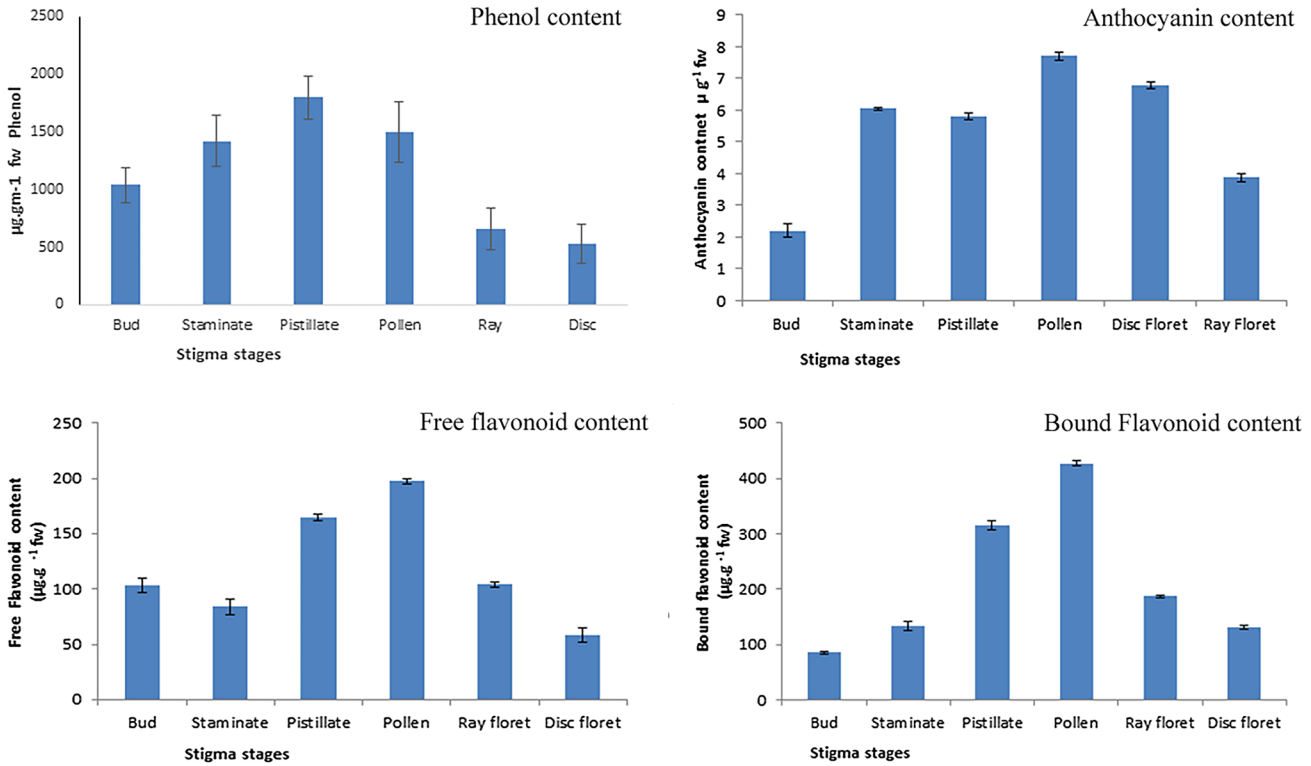


**Fig. 2** Spectrophotometric analysis of the components of inflorescence-total phenol content, anthocyanin content, free flavonoid content and bound flavonoid content

## 4 Discussion

**High anthocyanin content in pollen, stigma and the disc florets may aid in attracting pollinators in sunflower** – Three main types of pigments provide colours in plants—anthocyanins, chloroplast containing pigments, chlorophyll and carotenoids, and betalains. Anthocyanins are group of flavonoids that provide red, violet and blue colours to leaves, stem, flowers and fruits of higher plants. Anthocyanins are derived from phenylalanine, water-soluble pigments, synthesized in the cytoplasm of plant cells and then sequestered into vacuoles by glutathione pumps. The variation in the colour that ranges from red to violet–purple depends on the structure of anthocyanins, metal ion associations, co-pigments, and vacuolar pH (Tanaka et al. 2008). Most of the plants develop more anthocyanin during reproductive phase. Anthocyanins aid the plants in attracting or repelling different pollinators and seed dispersers, protect the plants against abiotic stress like ultraviolet radiation, temperature variation, and defence against various pathogens, insects, and herbivores (Anderson et al. 2010). Present investigations reveal higher anthocyanin content in the pollen, disc florets and the staminate stage of stigma which may attract the pollinators. In different plants, such as *Trachystmeon* sp., anthocyanin accumulation has been linked with the specific stage of development. The variable content of anthocyanin in the bud and staminate stage of stigma development may also point to their role in the development of stigma. A high anthocyanin content in pollen, disc florets and staminate stages of stigma may also be induced by light as has been observed in *Chrysanthemum* sp. (Hong et al. 2016). The yellow colour in the inflorescence of sunflower is due to the presence of water repelling pigment carotenoids (beta-carotenes), produced in the chromoplasts of sunflowers. These pigments absorb blue wavelength and scatter the longer wavelengths, producing yellow colour.

**Phenols and flavonoids act as antioxidants during stigma maturation** – Phenols are aromatic benzene ring compounds with one or more hydroxyl group substitution. Phenols are synthesized in chloroplasts and are often stored in the vacuoles of subepidermal cells of plants that are exposed to stress. Phenols have diverse roles in plants, which include lignin and pigment biosynthesis, providing structural integrity, protection against UV radiation, free radical scavenging, and they provide both active and passive resistance by repelling/killing different micro-organisms. The concentration of phenols may vary due to various internal and external factors, including trauma, injury, pathogen attack, drought, photoinhibition, nutrient stress and photo-protection (Younis

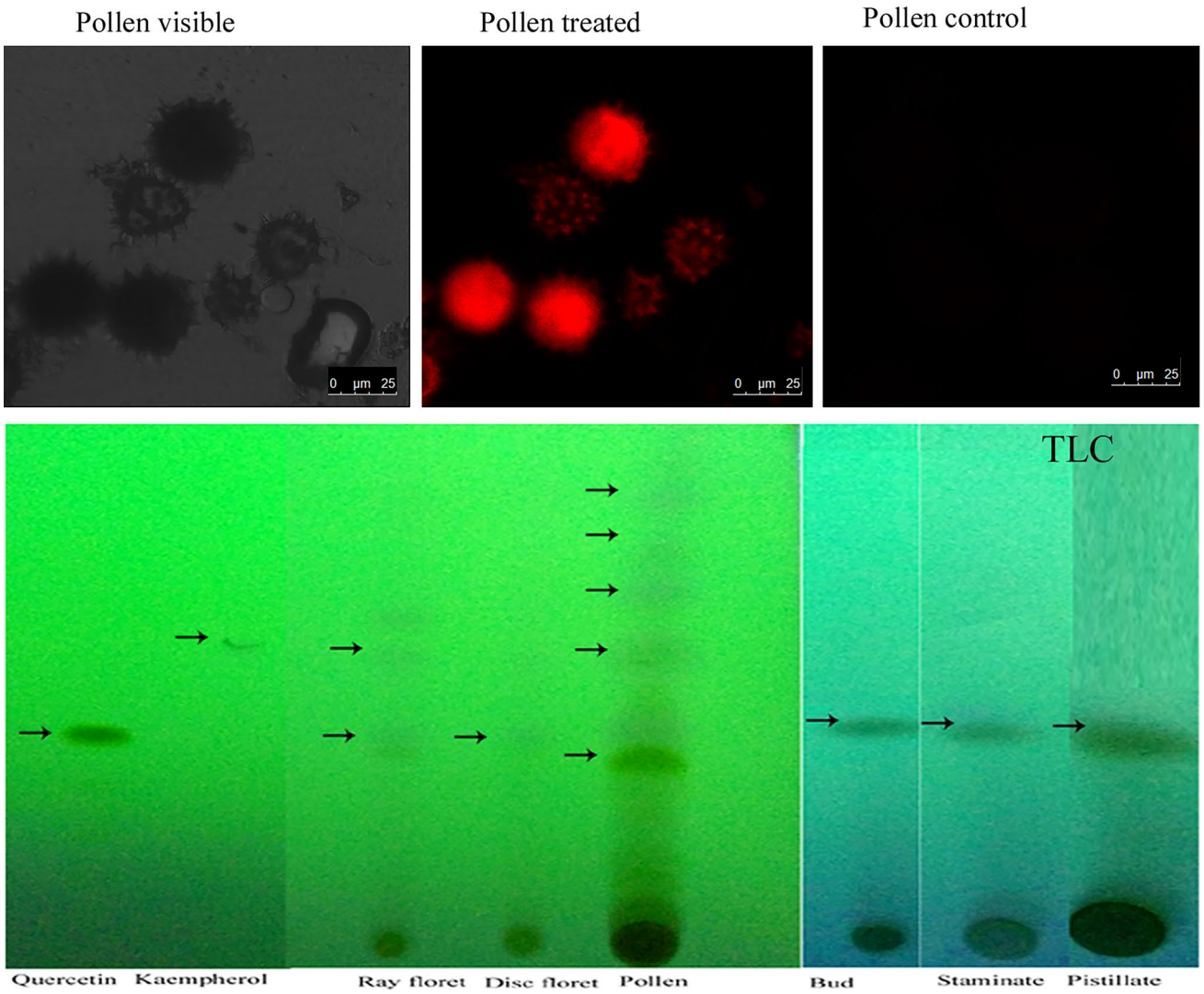


**Fig. 3** Confocal images showing the localization of flavonoids by fluorophore diphenylboricacid 5,2-aminoethylester (DPBA) in pollen (×400) in visible treated and control situations. TLC image showing identification of flavonoids in the inflorescence of sunflower

et al. 2010; Bhattacharya et al. 2010). Plant phenolics and flavonoids are involved in defence against biotic and abiotic stresses. Higher phenolic compound in the capitulum has been known to provide resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary (1884) (Prats et al. 2003). Many researchers have pointed out that the antioxidant capacity of plants is influenced by the phenolic content, flavonoid content and the stage of maturation or development (Stylar et al. 2016). Under normal physiological conditions, there is a production of free radicals from the chloroplasts, mitochondria and peroxisomes, and relatively small amount of antioxidants are sufficient to maintain redox homeostasis. Plants, however, are sensitive to abiotic stress, including high light intensity, drought, and biotic stress like pathogen, which lead to threefold to tenfold increase in free radical concentration, and consequently increase in antioxidant concentration (Kasote et al. 2015). Present investigations reveal that phenol content increases as the stigma matures. The total phenol content in the various components of inflorescence is higher than that of total flavonoid content revealing that phenols play a major role as antioxidants. Earlier studies have pointed out that there is nectar secretion and ejection of the pollen in the staminate stage of stigma, which leads to an increase in the visit of pollinators. This new interaction causes stress in the pollen and stigma. Also, the receptive stigma has to differentiate between the suitable pollen and the microbe, which leads to increase in the reactive oxygen species (Sharma and Bhatla 2013a, b, 2014) resulting in the increased antioxidant concentration.

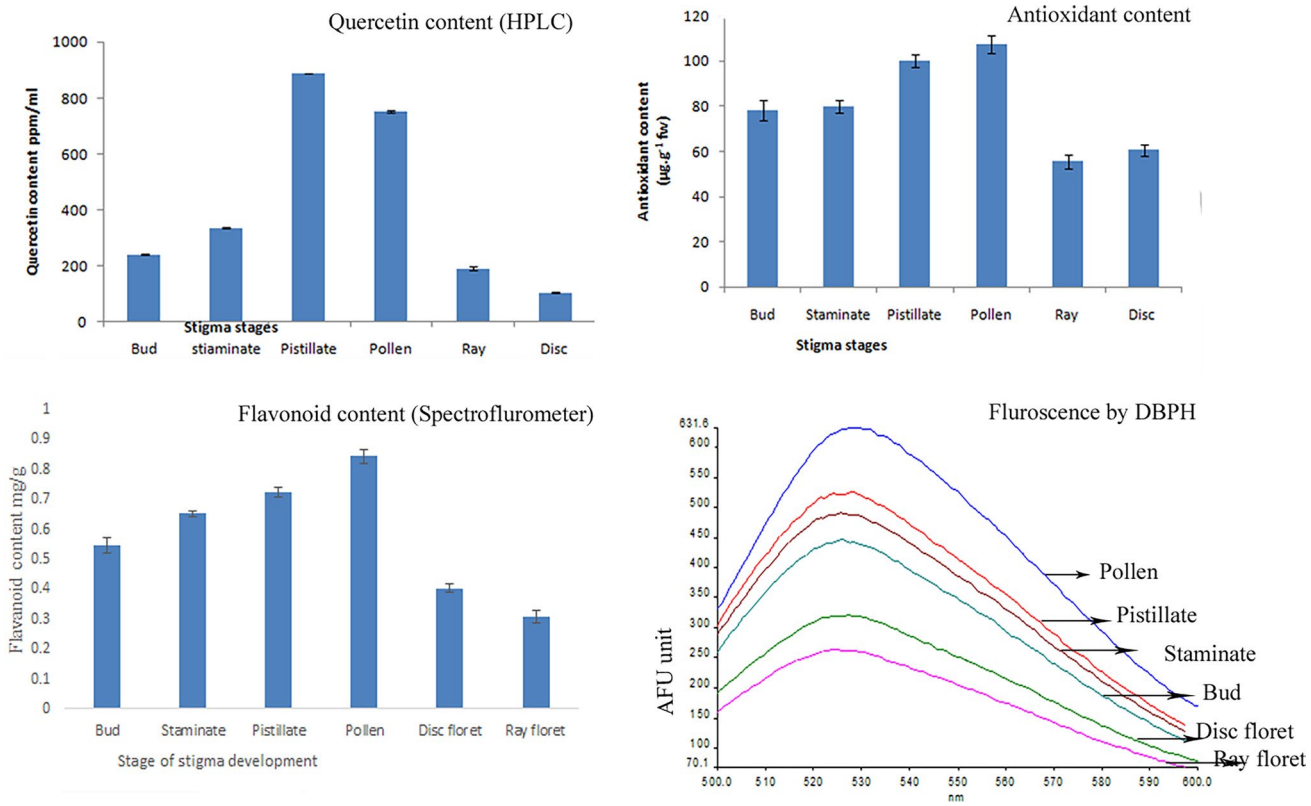


**Fig. 4** Antioxidant content in inflorescence of sunflower, quantification of flavonoid by RP-HPLC, spectrofluorometric analysis in the various components of sunflower inflorescence

**Bound flavonoid content may have a role in stigma and pollen maturation in addition to inhibiting pathogens** – Flavonoids are secondary metabolites, not essential for the survival of plants. They, however, have an essential role in plants, including colouration of flowers, as pollinator attractants, protection of plants form microbes and insects, detoxifying agents, stimulants for germination of spore, seed germination, UV filters, allelochemical agents and as signalling molecules. Flavonoids are produced in the cytosol of cells and are accumulated in the vacuoles. They are associated with lipophilic compounds in epidermal cells or form exudates of the roots. Flavonoids may occur in free state or may be bound with sugars at C3 positions (Skerget et al. 2005; Kumar and Pandey 2013). It has been reported that in *Terminalia arjuna* (Roxb.) Wight and Arn (1834) bound flavonoids exhibit higher antimicrobial activity (Jaiswal and Kumar 2015). Flavonoid aglycones are more potent in their antiperoxidative action than their corresponding glycosides (Das and Pereira 1990). Glycosylation of flavonoids increases their solubility in aqueous media and reduces chances of OH group from auto-oxidation. Flavonoid aglycones also allow the transport of flavonoids from endoplasmic reticulum to other cellular compartments and help in the secretion of flavonoids (Pollastri and Tattani 2011). Present investigations reveal that both free and bound flavonoid content is higher in receptive stigma and mature pollen, when they are exposed to biotic stress. Flavonoids also have the capacity to absorb solar energetic wavelength (UV A, B), thereby saving the plants from harmful solar radiations. They also inhibit the generation of reactive oxygen species and quench ROS once they are formed, acting as detoxifying agents (Brunetti et al. 2013). The presence of flavonoids in the various components of the inflorescence shows their diverse functions in sunflower. The epidermal cells of the ray florets have a reflective distal tip and UV absorbing base, due to different carotenoids and flavonoid pigment in cytoplasm and vacuoles of epidermal cells. UV reflecting distal parts acts as long distance recognition of inflorescence, and the UV absorbing basal part acts as honey guide towards nectar and pollen in the disc floret (Sammataro et al. 1985). Flavonoids are also known to be stored in tapetosomes, which are present in the tapetum cells of the developing pollen cells. They are known to accumulate oleosin-coated lipids and ER derived flavonoids which are transferred on the pollen coat at maturity (Hsieh and Huang 2007; Fambrini et al. 2010). The flavonoids protect the nucleic acid against UV damage (Pacini and Hesse 2005; Song et al. 2015). In some plants like *Zea mays* L. 1758 and petunia (*Petunia hybrid* Vilm

1863) flavonoids in the pollen coat are involved in hydration, pollen tube germination and growth (Mo et al. 1992; van der Meer et al. 1992; Napoli et al. 1999). Similar to the present investigations, in *Brassica napus* L. (1753), quercetin and kaempferol are the major flavonoids identified. Quercetin and kaempferol are characterized by high antiradical activity (Hsieh and Huang 2007; Ceksteryte et al. 2016). In *Carya illinoinensis* (Wangenh) Koch (1869) flavonoid incorporation could improve in vitro pollen germination (Wood 2017). Flavonoid composition of stigmatic surface may also affect pollen germination (Rejón et al. 2014).

**Flavonoids may act as signalling molecules** – Flavonoids have been reported to act as signalling molecules. Morphological, biochemical and anatomical studies in stigma have revealed ER-Golgi exocytic pathway contributing to stigmatic exudate and their role in pollen recognition and hydration (Rejón et al. 2014). Molecular studies in *Arabidopsis thaliana* (L.) Heynh (1842) have revealed the presence of Flower flavonoid transporter (FFT AtDTX35) in the floral epidermal cells of stigma, anther and nectaries. Flavonoids are present in the epidermal cells and affect metabolic activities like pollen development and release. Studies on mutant of *Arabidopsis* devoid of chalcone synthase revealed that the tapetum tapetosomes and pollen coat do not contain flavonoid. Their pollen is shrunken/having irregular surface (exine) with reduced viability (Thompson et al. 2010). FFT (flower flavonoid transporter) transcript was also seen in the nectar, coinciding with the initiation and decline of anthesis. Earlier studies in sunflower have also revealed an ER-Golgi exocytic pathway which is associated with flavonoid synthesis (Sharma and Bhatla 2013b). Flavonoids in stigma show a role in combating heat stress, pollen recognition, pollen hydration or generation of signals for the growth of pollen tube towards the style. It has been suggested that flavonoids regulate auxin transport in the pollen tube (Buer and Muday 2004). Interplay of ROS (in mature stigma) and nitric oxide (in pollen) is known to occur during pollen–stigma interaction (Sharma and Bhatla 2013a). Studies in maize have proved that concentration of flavonoids and nitric oxide increases when stimulated by UV-B radiations. Leaves pre-treated with NO scavenger PTIO do not accumulate NO and flavonoids, showing that flavonoids have some role in the NO-dependent signalling pathways (Tossi et al. 2012). Flavonoids also take part in flavonoid-peroxidase reaction mechanism for scavenging $H_2O_2$, thereby playing a role in the signalling mechanism through ROS. Flavonoids interact with cellular receptors or proteins (kinases and enzymes) involved in signalling regulation of physiological response or gene expression (Mattson 2004; Williams et al. 2004). Recent investigations have revealed the ability of flavonoids to interact with MAPK (Mitogen activated protein kinases) which affect cell growth and differentiation (Brunetti et al. 2013).

In this study, the presence of flavonoids, their quantification and localization were monitored in the various components of sunflower inflorescence. Flavonoids help in the attraction of pollinators by providing colour and aroma in the form of anthocyanin. High levels of phenols and flavonoids, both in free and bound forms, act as antioxidants and protect the various components of inflorescence against biotic and abiotic stress. The localization of flavonoids on the pollen coat reveals their pivotal role in reproductive biology of sunflower. Further studies are required to know the molecular and role of flavonoids and their interplay with signalling molecules in pollen–stigma interaction and pollen germination.

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**Author contributions** Basudha Sharma has contributed in the form of designing, carrying out of experiments, data analysis and writing of the paper. External help has been taken from different institutes in the form of instrumental facilities.

**Complaince with ethical standards**

**Conflict of interest** The author declares that there is no conflict of interest.

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