ISSN: 2287-688X

Annals of
Plant Sciences

Volume 10, Issue 12(2021) pp. 4377-4387

Research Article

Phytochemical Analysis with Quantitative Evaluation of Protein of Floral Maize Pollen

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Abstract

Bee pollen is commonly used as a dietary supplement and is recognized for its medicinal properties. Floral pollen is less recognized due to a lack of investigation. This study aims to determine the morphological characteristics and nutritional and phytochemical properties of floral maize pollen. The main composition of floral maize pollen is carbohydrates (64.15%), followed by moisture (5%), crude proteins (15.75%), crude fibers (7.4%), and ash (3.3%), while the lowest content is observed for crude fats (4.4%). Excellent phytochemical properties add value to floral maize pollen. Maize pollen possesses strong antioxidant activity. The acetone extract exhibited the highest radical scavenging activity with 83.97±0.04 % followed by its methanol extract with 80.20±0.09, ethanol extract with 66.20±0.06 and aqueous extract with 33.73 % by DPPH assay. In overall comparison of different extracts the Acetone extract of *Zea mays* (*saccharata*) show the highest scavenging activity followed by the methanol, ethanol and then aqueous extract. Acetone and Methanol has been proven as effective solvent to extract phenol compounds. Maize floral pollen and derived products can serve as future food resources for human consumption and as a source of functional and bioactive compounds in nutraceutical and pharmaceutical industries.

Keywords: *Zea mays (saccharata), pollen, phytochemicals, proteins, antioxidant activity*

Introduction

Maize or corn (*Zea mays L.*) is a plant belonging to the family Poaceae. It is a monoecious and annual plant grown widely all over the world. There are different types of maize, e.g., feed corn (*Zea mays* var. *indenata*), flint corn (*Zea mays* var *indurata*) ,dent corn , flour corn ,popcorn , waxy corn, high-amylose corn and sweetcorn (Zea mays var. saccharata) All parts of maize plants are useful, such as food and feed for humans and livestock, respectively. Maize cobs provide a soft-grit abrasive and furfural. Extracted oil, bran and starch come from the plant kernel [Naves, M.M.V. *et al*., 2011]. The silk of maize is used for animal feed and silage. Maize husks are filling materials for dolls, whereas paper and wallboard come from the stalk of maize plants [Ranum, P. *et al*., 2013]. Male gametophytes of plant seeds produce pollen grains [De- Arruda. *et al*., 2013]. Pollen grains are living organisms and both the environment and genotype influence their behavior and survival. Pollen grains have various shapes, sizes and surfaces. They possess nutritionally essential substances, such as carbohydrates, proteins, amino acids, lipids, and mineral substances [Rzepecka-Stojko, A. *et al*., 2015]. Significant amounts of phytochemical including carotenoids, steroids, terpenes and flavonoids, are present in floral maize pollen [De- Arruda. *et al*., 2013; –Aličić, D. *et al*., 2014]. Pollen is used in pollination and as a food for insects [McQuate, G.T. *et al*., 2003]. In addition, pollen has gained attention for its therapeutic properties, such as its antibacterial [Garcia, M. *et al*., 2001; Domenici, V. *et al*., 2015], anticariogenic [Almas, K. *et al*., 2001] and immunomodulatory effects [Gebara, E.C. *et al*., 2002]. For centuries, apicultural products have been used in phytotherapy and diet due to their positive health implications [Rzepecka-Stojko, A. *et al*., 2015; Aličić, D. *et*

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DOI: http://dx.doi.org/10.21746/aps.2021.10.12.3

al., 2014; Kocot, J. *et al*., 2018; Yang, Y. *et al*., 2020]. Bee-gathered pollen (bee pollen) is an apicultural product of great commercial interest due to its high nutritional value and physiological properties, representing an important energy and protein source for human nutrition. Considering the positive effects of floral pollen nutrients and phytometabolites on human and animal health, floral pollen can serve as a future food resource and a source for product derivation [Kostic, A.Z. *et al*., 2020]. According to statistics on maize production by the United States Department of Agriculture (USDA), the largest producer of the maize crop is the United States of America, with 347,782 tons in 2019, while Malaysia produced 58 tons of maize crops. Floral maize pollen was selected in this research due to its ample amount produced during anthesis. Therefore, instead of wasting these products, this research aims to investigate the utilization of useful maize pollen products as dietary supplements for human health. For the above purpose, we assessed the nutritive properties and antioxidant activities of floral maize pollen.

Materials and Methods

Sample Collection and Storage

Zea mays plant variety sweet corn was planted on field area of Vidya Pratishthan's College of Agricultural Biotechnology, Baramati. Fresh floral maize pollen was collected during anthesis from plants randomly selected from the planted maize plants. Pollens were collected into separate Ziploc bags by gently tapping the main stems of the maize plants. The Ziploc bags containing floral maize pollen were brought immediately to the laboratory. The floral maize pollen was cleaned, shifted through a sieve and placed into an airtight container. The pollen, either fresh or stored at -20˚C, was subsequently used for the various analyses described below.

Pollen Morphological Observation

The detailed morphological structures of the anther, fresh and dehydrated pollens were examined under a compound microscope and their sizes were recorded. The collected pollen was then characterized by microscopic examination for identification of the botanical origin of the pollen under 40X and 100X magnification.

Proximate Analysis of the Floral Maize Pollens

Proximate analysis of the moisture, ash content, crude proteins, crude fats, and crude fibre composition of floral maize pollen was determined using the standard methods of the Association of Official Analytical Chemists (1990). The moisture content of the pollen samples was determined by drying each sample until a constant weight was obtained. The ash value was determined by incinerating air-dried samples in a muffle furnace at 550˚C for 5–6 hours [AOAC, 1990]. The percentage of crude protein content was determined by multiplying the percentage of nitrogen content obtained from the samples using Kjeldhal Method by a factor of 6.25 [AOAC,1990]. The crude fibre was estimated by acid-base digestion based on [AOAC, 1990] method.

The standard method of AOAC (1990) was used and the carbohydrate content of the samples was determined by difference as follows. % carbohydrate= 100-(% moisture+%protein+%fat+%crude fiber)

Analysis of Secondary Metabolites by Standard Protocols

Extract Preparation Suspend 2 gram of fresh pollen in 20 ml of solvent at RT for 72 hrs on rotary shaker. Centrifuge the sample at 5000 rpm for 15 mins. Filtered the supernatant through Watman's No 1 filter paper. The supernatant was then collected and filtered. This liquid extract was then dried and concentrated in a rotary evaporator, under reduced pressure at less than 40°C, to get respective type of extract. The extract was collected and stored at -80°C until further analysis.

Analysis of Secondary Metabolites by Standard Protocol-

The secondary metabolites in the plant sample are the main concern of research

work. There are many tests available for this purpose.

Qualitative Phytochemical Analysis:-
The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standards procedure.

Biochemical or Phytochemical Tests [Sawant, R.S. *et al*., 2013]

- **Tannin** 4ml extract was treated with 4 ml $FeCl_3$ formation of green colour indicates that presence of condensed tannin
- **Saponin** 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin.
- **Anthocyanin**2 ml of aqueous extract is added to 2 ml of 2N HCl and NH_3, the appearance of pink red turns blue violet indicates presence of Anthocyanin.
- **Coumarin** 2ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.
- **Alkaloids** A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixturewas heated gently for 20 min cooled and filter, the filtrate was used for following test. Wagner test: Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.
- **Flavonoid:** Alkaline reagent test: Extract was treated with 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid. The filtered extracts were used for the determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA).
- **Phenolic compound** Add 3-5 drops of 5% $Fecl_3$ solution to 2ml extract
- **Phlobatanins** 2ml of extract to 2ml of 1%HCL and boil the mixture.
- **Leucoanthocyanin** Add 5ml of aqueous extract to 5ml of isoamyl alcohol.
- **Terpenoids** Add 2ml of extract to 2ml of acetic anhydride and Conce. H_2SO_4.
- **Steroids** Dissolve 1ml of the extract in 10ml of chloroform and add equal volume of concentrated sulphuric acid by sides of the test tube
- **Fatty acid** Mix 0.5 ml of extract with 5ml of ether, allow these extract for evaporation on filter paper and dry the filter paper.

Isolation of Protein Extract and Quantification of Total Protein [Talukdar, G. *et al*., 2012**]**
Ammonium Sulphate Precipitation Collected crude supernatant of sample was taken for 20% cut off. According to the salt chart ammonium sulphate was measured and thoroughly ground by mortar pestle to make a soft amorphous salt. Then it was added to supernatant with a continuous stirring. After addition of the salt the sample was stored in 4°C for overnight incubation. Next day the sample was centrifuged and supernatant was collected again for the next cut off i.e. 40% cut off. Supernatant collected, was measured and salt is added according to the salt chart. Perform the same procedure for 60% and 80% cut off.

Dialysis The membrane was prepared according to instructions. Sample was loaded into dialysis tubing, cassette or device. Sample was placed into an external chamber of dialysis buffer (with gentle stirring the buffer).Dialyzed for 2 hours (at room temperature or 4 °C).The dialysis buffer was changed and dialyzed for another 2 hours.

Quantification of Proteins by Using Nanodrop Spectrophotometer [Gupta, A. *et al*., 2017**]**
Clean the upper and lower optical surfaces of the micro spectrophotometer.

Pipette 1-2µl of clean deionized water onto the lower optical surface .Close the level arm and tap it a few times to bathe the upper optical surface. Lift the level arm and wipe off both optical surfaces with Kimwipe. Open the Nanodrop software and select the protein module.

Initiate the spectrophotometer by placing 1µl clean water onto the the lower optic surface,

lowering the level arm, selecting "initialize "in the nanodrop software. Once initialize completes, clean both optical surfaces with Kimwipe. Perform a blank measurement by loading 1µl deionized water or buffer and selecting blank .once blank is complete ,clean both optical surfaces with Kimwipe. Measure the protein sample by loading 1µl and selecting "measure". Once measurement is complete clean both optical surfaces with Kimwipes.

Determination of Antioxidant Activity (AA)

The antioxidant activity was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method based on quantifying the free radical scavenging activity of the extracts described by [Gupta, A. *et al*., 2017]. At first 4 tubes for each extract were taken to make aliquots of 20%, 40%, 60% and 80% concentration with the sample extract. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogenously magnetic stirrer was used. After making desired concentrations 3 ml of 0.004 % DPPH was applied on each test tube by pipette.

Record the room temperature and kept the test tubes for 30 mins in light for complete reaction.

After 30 min absorbance were taken at 517nm on spectrophotometer and ethanol + DPPH solution was used as control.

Results

The release of pollen grains can start from sunrise until noon depending on the plant's temperature, humidity and genetic constitution [Kaefer, K.A.C. *et al*., 2016]. The pollen was harvested as soon as anthesis occurred during the 6th and 7th weeks after planting at approximately 9.30 am to 12.30 pm. As the anthers of the *Z. mays* plant dehisce, they split apart to allow pollen grain to fall into the open air. A tassel typically sheds pollens for approximately five days. Pollen shed in a field can last up to two weeks. The size of shrunken pollen is 9.87±0.38 µm (range from 9.52 to 10.81 µm) x 8.11±0.77 µm (range from 6.74 to 9.18 µm) as per the (Fig. 2 A and B). The fresh pollen shape changed from a prolate spheroid to an indented, prismatic solid (Fig 1 A) and changed colour from yellow to amber when dehydrated. The pollen is yellow due to its main flavonoid, quercetin [Freire, K.R. *et al*., 2012]. High temperature and low humidity of the environment shrinks maize pollen. Maize pollen is very sensitive to high temperature and desiccation. The shrunk pollen resembles the seeds of the maize itself (Fig 1 B). According to Aylor [Aylor, D.E. *et al*., 2003], floral maize pollen is sensitive to dehydration and rehydration. The deterioration of pollen during storage and drying involves many physical and chemical changes, including changes in odour, taste, colour, and shape, disrupted intracellular integrity, decreased enzyme activities, lipid peroxidation and phenolic oxidation.

Collection of Pollen

Pollen was collected from *Zea mays* plant in the period of month of February 2021. About 30 g of pollen was collected during this period.

Morphological Characteristics of Floral Maize Pollen

Fig 1(A, B, C, D) Morphology of floral maize pollen under Compound Microscope (magnification 200x)

Palynological Study of Zea mays (Saccharata) (Inyana CN *et al*.)

It has been reported that palynological study after acetolysis gives much clear features under compound microscope (Figure 1 A, B, C, D) with acetolysis, without acetolysis, with saffranin staining and are very useful in distinguishing closely related species of *Zea mays* has a perforate sculpturing of pollen exine with a few unevenly distributed holes or depression, while rest of the surface of pollen is psilate (Table 1).

Sl. No	Features	Observation Result
1	Size	0.07 mm(7µm) at 10 x
2	Shape	Circular elliptical
3	Aperture	pore
4	Exine sculpture	Reticulate (coarse)
5	Exine thickness	Medium with rods

6	Polar view	Circular

Characteristic Features of Pollen (Table 1)

Fig. 1(A)

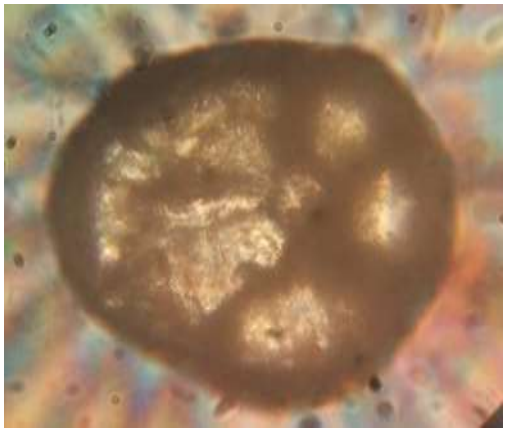


Fig. 1(B)

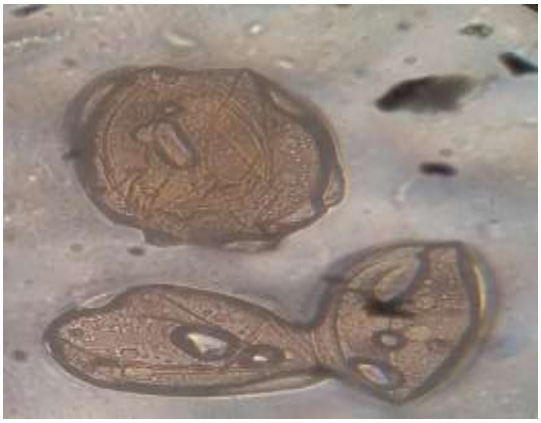


Fig. 1(C)

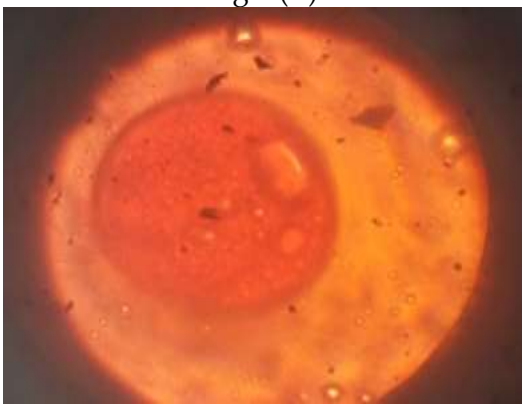


Fig. 1(D)

Fig. 1(A) and Fig. 1 (B) are the microscopic observations without acetolysis at 40x and 100x magnification respectively.

Fig. 1 (C) is a compound microscopic observation after acetolysis at 40x magnification.

Fig. 1 (D) is a microscopic observation staining with saffranin at 100x magnification.

Measurement of pollen with micrometer lens

Fig 2(A). Zea mays pollen under 40x

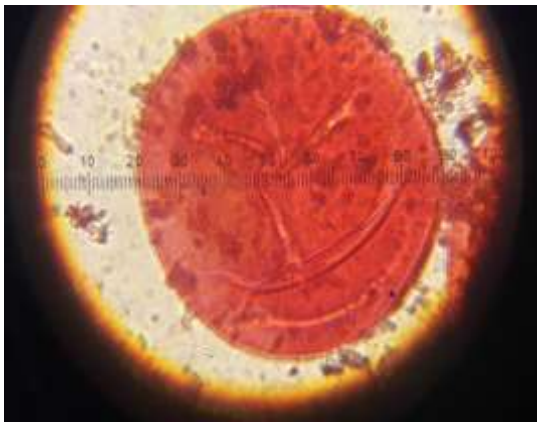


Figure 2(B). *Zea mays* pollen under 100 X

Proximate Composition of Floral Maize Pollen

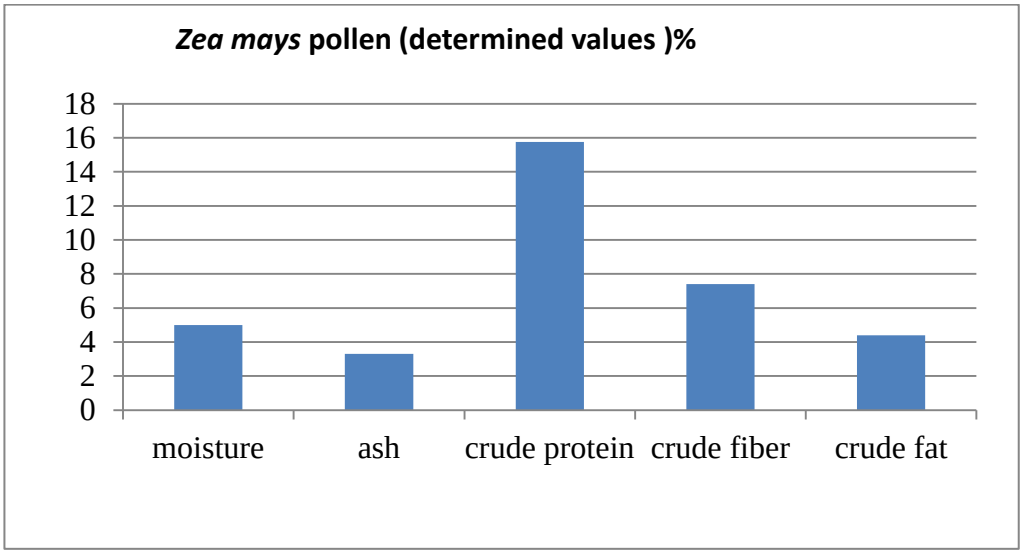
Table 2: Proximate Analysis

Sl. No.	**Compounds**	***Zea mays* pollen (values) %**
1	Moisture	5
2	Ash	3.3
3	Crude protein	15.75
4	Crude fiber	7.4
5	Crude fat	4.4
6	Carbohydrates	64.15

Table 2 Shows the proximate composition of floral maize pollen and other floral pollens. Categorically, the proximate composition of maize pollen is represented as carbohydrates > crude proteins > crude fibres > moisture>crude fats> ash.

This study was done to know the nutritive value of the *Zea mays* (saccharata) pollens sample. Using standard procedures, the proximate composition of the Zea mays (saccharata) pollens were determined and presented in Table 2. The Table 2 showed that proximate analysis pollens, it contained ash (3.3%), crude protein content is (15.75%) and, moisture content is (5%).crude fiber content is (7.4%), crude fat content is (4.4%) and carbohydrate content is (64.15%) The result obtained as per the Graph No. 1 showed that Zea mays pollen has high protein and carbohydrates content. It has low in moisture contents which is increases longetivity of pollen for storage.

Moisture content is vital to ensure the stability and quality of pollen. Fresh and dry pollen loads have different water contents, ranging from 20–30% in the original form and 4–10% if dried, affecting organoleptic and "shelf lifetime" properties [Edeoga, H.O . *et al*., 2005].



Graph No. 1: proximate analysis

Phytochemical Analysis from *Zea mays*

Fig. 3 A

Fig. 3 B

Phytomedicine represents one of the most important fields of traditional medicine all over the world and are of prime importance to the health of individuals and communities. The medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological

action on human body like alkaloids, tannins, flavonoids and saponin etc. [Pascoal, A. *et al*., 2014].

Phytochemical Analysis

Table 3

Phytochemicals	Inference					
	D/W	E	M	C	P	A
Alkoloids	+	+	+	-	+	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Saponins	-	+	-	-	+	-
Tannins	+	+	+	+	+	+
Philobatanins	+	+	+	+	+	+
Coumarins	-	+	-	-	+	-
Anthocynins	+	-	-	-	-	-
Leucoanthocynins	+	-	-	-	-	-
Terpenoids	-	+	+	+	+	+
Steroids	-	+	+	-	+	+
Fatty acids	-	+	+	-	-	-

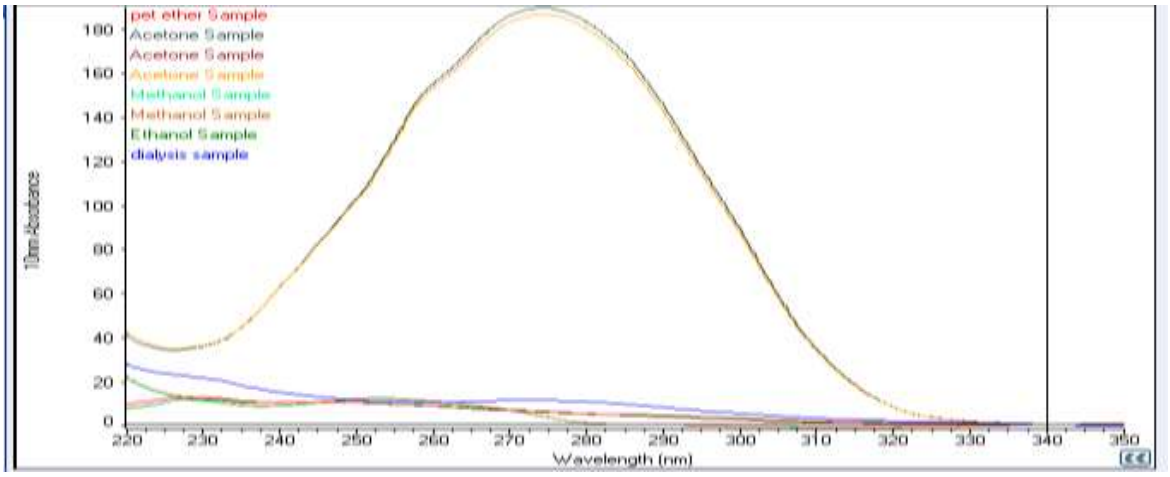
Table: + presence, - absence

D/W=Distilledwater C=Chloroform E=Ethanol, M=Methanol,A-Acetone

Phytochemical analysis as per Table 3 and Figure 3 A and Figure 3 B revealed the phytochemicals alkaloid, phenolic compounds, terpenoids, flavonoids, tannins, saponins, steroids are present .these compounds are isolated by me are known to be important in medicinal sciences. Terpenoids have analgesic properties and known to decrease blood sugar level. Flavonoids are water soluble antioxidant and it shows various biological activities like anti-microbial, anti-allergic and anti-inflammatory in the pollen extract of *Zea mays*. Tannins: Are reported to have biological activities like anti-tumor and anti-bacterial.

Quantification of protein by Nanodrop from *Zea mays*

The NanoDrop ND-1000 spectrophotometer uses a patented sample retention system that holds 1 µl of sample without the need for traditional containment devices such as cuvettes and capillaries. Using fiber optic technology and surface tension, the sample is held in place between two optical surfaces that define the path length in a vertical orientation. Removal of fixed containment devices from the system allows the path length to change in real time for a given sample. This essentially eliminates the need to perform dilutions or to make any assumptions regarding the sample concentration prior to the measurement. Direct coupling of the sample to the optics of the spectrophotometer removes interference caused by incident light and transmitted light passing through containment walls of traditional curettes, microcell curettes, and capillaries. Preparation for the next sample only requires wiping of both optical surfaces with a common laboratory wipe. The time needed for performing dilutions and for cleaning the cuvette and microcell devices, as well as the possibility of damaging such devices, is eliminated. Total measurement cycle time, including preparation and removal of the sample, is -30 sec. The ease of use of this protocol not only makes it a feasible option for small volume analysis, but also a practical alternative for all spectrophotometric measurements.

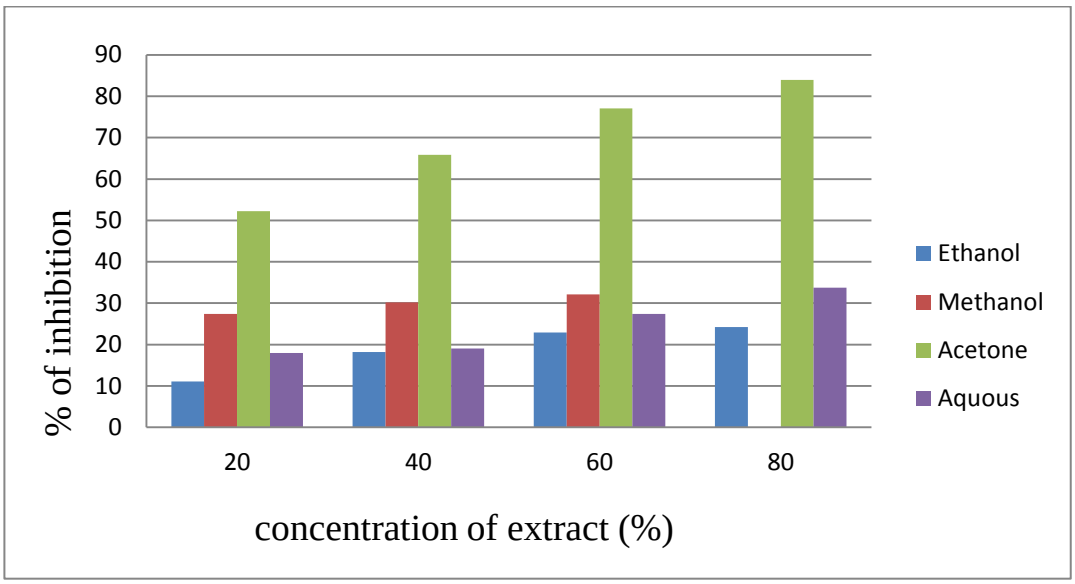


Graph No. 2: Quantification of protein

Protein quantification of pollen extracts was done by using nanodrop as shown in above Graph No. 2 the crude protein acetone extract shows highest protein concentration of **181.885** mg /ml and purified PBS extract showed **18.28** mg/ml is also high in protein than reported value in corn silk is 6.50 mg/ml.

Antioxidant Activity by DPPH Assay of Different *Zea mays* Pollen Extract

Anti-oxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, and ischemia injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). [Donald, R.B. *et al*., 1987]



Graph No. 3: Antioxidant activity of sample in diffent solvents

Graph No. 3 shows the results of the free radical (DPPH) scavenging activity in terms of % inhibition. The result revealed that the acetone *extract* exhibited the highest radical scavenging activity with 83.97±0.04 % followed by its methanol extract with 80.20±0.09, ethanol extract with 66.20±0.06 and aquous extract with 33.73 %. In overall comparison of different extracts the **Acetone** extract of *Zea mays (saccharata)* show the highest scavenging activity followed by the methanol, ethanol and then aquous. Acetone and Methanol has been proven as effective solvent to extract phenolic compounds. In

the present study, the values of ethanolic and aqueous extracts were higher than Petroleum Ether. Among solvents used in this study **Acetone** has showed the best effectiveness extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because it's low toxicity. Graph shows the antioxidant activities of *Zea mays* (saccharata) pollen in different solvent extract.

Discussion

Palynological study gave clear microscopic image and its morphological characteristics. Qualitative phytochemical screening indicated presence of secondary metabolites that are essential in herbal medicines. Among the phytochemicals obtained where alkaloids, tannins, phenolics, saponins, Coumarins, etc. Proximate analysis shows the presence of nutritional compounds mainly carbohydrates, proteins etc. Phenol and tannins that act as primary antioxidants or free radical scavengers. Gives antioxidant activity by DPPH Assay from ethanol, Methanol and acetone extract of assays shows high antioxidant activity than the aqueous extract. Protein quantification of pollen extracts was done by using nanodrop, the crude protein acetone extract shows highest protein concentration of **181.885** mg /ml and purified PBS extract showed **18.28** mg/ml is also high in protein than reported value in corn silk is 6.50 mg/ml.

Conclusion

Phytochemical qualitative analysis of Zea mays pollen shows presence of secondary metabolites and quantitative results shows the amount of phenolic and tannin content which is responsible for antioxidant activity and act as free radical scavengers. Proximate study of Zea mays pollen shows the presence of nutritional compounds. Carbohydrate is a major nutrient compound analysed during proximate analysis. Phenol and tannins that act as primary antioxidants or free radical scavengers and gives antioxidant activity by DPPH assay. Acetone, methanol and ethanol extract of assays shows high antioxidant activity than the aqueous extract. The aim of this study was to evaluate the protein content from pollen of *Zea mays*. The results of these studies showed that proteins from pollen support the nutritional value and is responsible for enhancing its immunity through nutrition.

Acknowledgments

We are grateful to Dr Bharat Shinde Sir, Principal, Vidya Pratishthan's Arts, Science and Commerce College, Baramati. I am thankful to my Guide Dr. A.B. Gawate, Principal, Shri Sant Gadge Maharaj College, Loha, and Dist.-Nanded, India. We are grateful to Annals of Plant Sciences to review and check the English in this paper. The authors also would like to thank the Academic editor and two anonymous reviewers, whose constructive comments and inputs significantly improved the article.

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Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:
Harugade, S., and Gawate, A.B. "Phytochemical Analysis with Quantitative Evaluation of Protein of Floral Maize Pollen". *Annals of Plant Sciences.*10.12 (2021) pp. 4377-4387.

DOI: http://dx.doi.org/10.21746/aps.2021.10.12.3