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A phytochemical study on selected varieties of Chilli from Balapar village in the Jam Kandorna Taluka of Rajkot district, Gujarat

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Abstract
The study aimed to investigate the phytochemical composition of selected chili varieties, specifically Capsicum annuum L. cultivars "Saniya" and "Reva," grown in Balapr Village, Jam Kandorna Taluka, Rajkot District. Chilis are known for their bioactive compounds, which contribute to their medicinal properties, nutritional value, and overall health benefits. The research focused on evaluating the presence of key phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds in these varieties. These compounds are responsible for the antioxidant, anti-inflammatory, and antimicrobial properties often associated with chili peppers. The study also explored the variation in phytochemical content between the two cultivars, aiming to identify the most promising variety for potential health applications. This research is particularly relevant in the context of the increasing demand for functional foods and natural remedies. Understanding the phytochemical profiles of locally grown chili varieties contributes to promoting their cultivation and potential utilization in traditional medicine and the food industry. The choice of this topic was driven by the growing interest in the pharmacological potential of chili peppers and the need for scientific documentation of their benefits, particularly for the local agricultural community in Rajkot District.

Keywords: Varieties, chillies, Gujarat, capsicum, Balapar, Rajkot

Introduction

Rajkot district is one of the 33 districts of Gujarat State which is located in the Saurashtra peninsula. It is located between 23°08′ North latitude and 70°20′ East longitude. The district occupies an area of 11203 km^2 with 14 Talukas and 616 villages in it (https://rajkot.nic.in/). Balapar is a village in Jamkandorna Taluka in Rajkot District of Gujarat State, India that is situated 14km away from sub-district headquarter Jamkandorna (tehsildar office) and 89km away from district headquarter Rajkot. The total geographical area of the village is 553.28 hectares. (https://villageinfo.in/gujarat/rajkot/jamkandorna/balapar.html). Agriculture is one of the main occupations in Rajkot district along with a significant number of villages, including Balapar. It is known for its semi-arid climate and fertile black soils that support a variety of agricultural crops. Among these, chilli cultivation has emerged as a prominent and economically important activity for many small and marginal farmers in the village. A wide range of traditional and locally adapted Chilli (*Capsicum annuum* L.) varieties are cultivated here that needs scientific documentation especially in terms of their phytochemical composition as they hold much potential for nutritional, medicinal and industrial applications.

The genus *Capsicum*, belonging to Solanaceae family, includes a diverse array of both sweet and hot chilli peppers, boasting a rich history that stretches back to approximately 6000-6500 BEC. These peppers have long served not only as popular culinary spices but also as vital components in traditional medicine, highlighting their significance in human culture and global cuisines over millennia. In contemporary society, Capsicum varieties hold considerable commercial value; it is estimated that around one-fourth of the world's population incorporates these peppers into their diets regularly (Barboza *et al*., 2022) [2]. In addition to being sold fresh, chili peppers are processed into a variety of products that enhance their culinary versatility. These include ground powders such as paprika and cayenne, flavorful sauces like salsa and hot sauce, and preserved forms such as pickles and chutneys. This transformation into diverse products not only caters to varying tastes and preferences but also expands their usability in kitchens around the world, making them staples in numerous culinary traditions (Carrizo *et al*., 2016; Parvez, 2017) [11].

Understanding the importance of this, the present phytochemical study was undertaken with the main objective of documentation of phytochemical profile of local genetic varieties. The present study would help in filling scientific gap especially from a lesser-studied region like Jam Kandorna Taluka. In the present work, qualitative and quantitative phytochemical analysis were carried out in two selected varieties of chilli - *Capsicum annuum* L. cultivar Reva and *Capsicum annuum* L. cultivar Saniya.

Materials and Methods

The material selected for the study purpose were the mature fruits of two varieties of chilli -*Capsicum annuum* L. cultivar Reva and *Capsicum annuum* L. cultivar Saniya.

There is difference in the amount and type of phytochemicals found in whole chilli fruits with seeds and fruits without seeds, due to the variation in phytochemical concentration across different parts of the fruit. Hence, for both the varieties under study, two different type of samples were collected – (i) Whole Mature fruits with seeds and (ii) Mature fruits after removal of seeds.

Fig 1: Mature fruits of Variety Reva

Fig 2: Mature fruits of Variety Saniya

1. Sample Collection & Preparation

Fig 3: Drying,

Mature fruits of two varieties of chilli *Capsicum annuum* L. cultivar Reva and *Capsicum annuum* L. cultivar Saniya were directly collected from Chilli fields in Balapar Village in Jam Kandorna Taluka of Rajkot District using random sampling method. Mature fruits of both the varieties were shade dried until all water molecules evaporated and plants were sufficiently dried for griding. Following drying, the plant components were finely powdered in a mechanical blender and stored in airtight containers that were appropriately labelled for future use (Yadav & Agarwala, 2011) [16].

2. Extract Preparation

Plant extracts were prepared by Hot water extraction method. 200ml of distilled water was added to 5g of dried, finely powdered plant material in a beaker. For 20 minutes, the mixture was heated on a hot plate at 30 to 40 degrees Celsius while being constantly stirred. The water extract was then passed through filter paper, and the phytochemical analysis was performed using the filtrate. When not in use, the water extract was stored in the refrigerator (Yadav & Agarwala, 2011) [16].

3. Phytochemical Analysis

Qualitative and quantitative Phytochemical analysis was done for the following bioactive compounds from the plant extracts prepared.

a. Qualitative Phytochemical analysis

The extract prepared was tested for the presence of bioactive compounds by using following standard methods (Sofowra, 1993; Trease & Evans, 1989; Harborne, 1973) [7, 13, 15].

Sr. No.	Test	Observation	Inference
A.	Test for Proteins:		
1.	Millon's Test: Crude extract mixed with 2ml of Millon's reagent	Appearance of white precipitates turns red upon gentle heating	Presence of Proteins
2.	Ninhydrin Test: Crude extract boiled with 2ml of 0.2% solution of Ninhydrin	violet colour appeared	Presence of amino acids and proteins
B.	Test for carbohydrates:		
1.	Fehling's Test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled	A brick red precipitate appeared at the bottom of the test tube	Presence of reducing sugars
2.	Benedict's Test: Crude extract when mixed with 2ml of Benedict's reagent and boiled	A reddish-brown precipitate formed	Presence of the carbohydrates

3.	Molisch's Test: Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube.	Appearance of a violet ring at the interphase	Presence of carbohydrate
4.	Iodine Test: Crude extract was mixed with 2ml of iodine solution	A dark purple coloration	Presence of the carbohydrate
C.	Test for Phenols & Tannins:		
1.	Ferric Chloride Test: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$	A blue-green or black coloration	Presence of phenols and tannins
D.	Test for Flavonoids:		
1.	Alkaline Reagent Test: Crude extract was mixed with 2ml of 2% solution of NAOH	An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid	Presence of flavonoids.
E.	Test for Saponins:		
1.	Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously.	The formation of stable foam	Indication for the presence of saponins
F.	Test for Glycosides:		
1.	Liebermann's Test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added.	A colour changes from violet to blue to green	Presence of steroidal nucleus, i.e., glycone protein of glycoside
2.	Salkowski's Test: Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently.	A reddish-brown colour	Presence of steroidal ring, i.e., glycone protein of the glycoside
3.	Keller-Kilani Test: Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4.	A brown ring at the interphase	Presence of cardiac glycosides
G.	Test for Steroid: (i) Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise.	A red colour produced in the lower chloroform layer	Presence of steroids
	(ii) Crude extract mixed with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture.	The development of a greenish coloration.	Presence of steroids
H.	Test for Terpenoids: Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentration H_2SO_4 was added and heated for about 2 minutes	A greyish colour	Presence of terpenoids
I.	Test for Alkaloids: Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture.	Turbidity of the resulting precipitate	Presence of alkaloids

b. Quantitative Phytochemical analysis:

Preliminary phytochemical screening from the aqueous extract of the samples was done following the method of Aiyegroro & Okoh (2010) [1] for the following compounds.

1. Total Phenolic Content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modification. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2Co3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed gallic acid equivalent (mg/g of extracted compound).

2. Total Flavonoid Content:

Aluminium chloride colorimetric method was used with some modification to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined form the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound).

Results

a. Qualitative Tests

The results of the qualititative phytochemical tests for the samples of mature fruits with and without seeds for Saniya and Reva Chilli varieties are summarised as shown in the Table-1. The results revealed the presence of various medicinally active compounds in samples for the two different varieties studied. It was observed that there was difference in the amounts of compounds in the samples of fruits with seeds and without seeds as well as among different varieties studied. From the various test performed for carbohydrates, it was found that reducing sugars were found to be more in Reva variety as compared to Saniya according to Fehling and Benedict's test. In both the cases, fruits without seeds showed more amount of reducing sugars as compared to those with seeds from the extract prepared. Molisch test, a general test for all types of carbohydrates confirmed more carbohydrate levels in Reva as compared to Saniya. Also, extracts made from fruits without seeds showed more amounts as compared to those with seeds. However, Iodine test gave negative result in all the cases indicating absence of starch.

Table 1: Qualitative phytochemical analysis

Test Parameter	Test Method	Saniya (Fruits without seed)	Saniya (Fruits with Seed)	Reva (Fruits without seed)	Reva (Fruits with Seed)
Carbohydrate	Fehling Test	++	+++++	++++	+++
	Benedict Test	++++	++	+++++	+++
	Molisch Test	++++	++	+++++	++++
	Iodine Test	-	-	-	-
Phenols & Tannin	Phenol & Tannin	+++++	+++	++++	++
Flavonoids	Alkaline Reagent Test	++	++++	+++	+++++
Saponins	Saponin Test	+	-	-	-
Glucoside	Salkowski Test	+++++	++++	+++	-
	Keller-Kiliani Test	+	-	-	-
Steroid	Steroid Tests	+	++	+	+
Terpenoid	Terpenoid Tests	-	-	-	-
Alkaloid	Alkaloid Tests	-	-	-	+

Ferric Chloride test conducted for the presence of phenolic and tannin compounds showed presence of these compounds in both the varieties with Saniya variety showing higher presence. Alkaline Reagent Test conducted for the presence of Flavonoids showed higher amounts in fruits with seeds for both the varieties. However, their presence was more in Reva variety as compared to Saniya variety. Foam Test done for the presence of saponins showed their absence in all the cases with a trace amount only in Saniya variety fruits without seeds. Salkowski test determining the presence of steroidal nucleus (part of cardiac glucosides) showed strong presence in case of Saniya variety as compared to Reva. Also, in both the cases fruits without seeds possessed a greater number of glucosides in comparison to fruits with seeds. Keller-Kiliani test determines the presence of cardiac glycosides, especially those containing 2-deoxy sugars. This test gave negative result in all the cases with a very small amount only in Saniya variety fruits without seeds. Steroid tests showed very weak presence in all the cases whereas terpenoid test did not positive result in all the cases. Alkaloid test showed a very mild presence in Reva variety fruits with seeds.

b. Quantitative Estimations

1. Total Flavonoid content

Fig-1 represents the bar graph showing the total flavonoid content (expressed in mg QE/mL) present in different parts (fruit without seeds and whole fruit) of two *Capsicum annuum* varieties: Reva and Saniya.

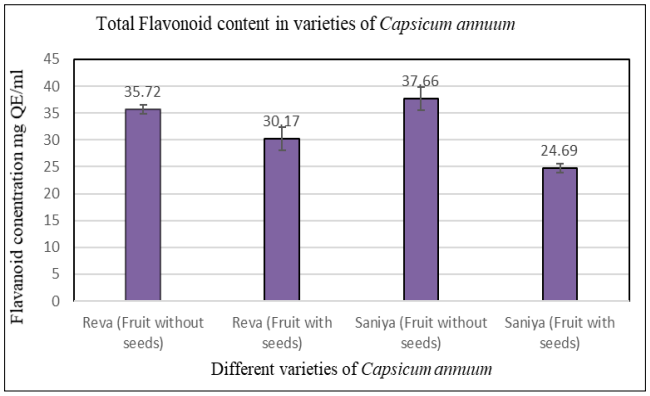

Fig 1: Shows the total Flavonoid contents of *Capsicum annuum* L. cultivar Reva and *Capsicum annuum* L. cultivar Saniya respectively

The highest flavonoid content was observed in Saniya fruits without seeds at 37.66 mg QE/mL, followed by Reva fruits without seeds at 35.72 mg QE/mL. A notable decrease in flavonoid concentration is seen in the seed-containing fruits. Seeded Saniya fruits showed the lowest flavonoid content of 24.69 mg QE/ml among all the samples. This trend suggests that a substantial portion of flavonoids is localized in the fruit pericarp or skin, rather than in the seeds.

In both varieties, the removal of seeds resulted in a significant increase in flavonoid concentration, indicating that the pericarp and surrounding tissues are the primary sites of flavonoid accumulation. The difference between fruits with and without seeds is more pronounced in the Saniya variety, suggesting a stronger flavonoid localization in the non-seed portions.

These findings reinforce the idea that seeds contribute little to flavonoid content, and that the edible flesh of the chilli fruit is the major source of these bioactive compounds. Given flavonoids' antioxidant, anti-inflammatory, and health-promoting roles, Saniya (without seeds) appears to be the most promising variety for nutraceutical and functional food applications.

2. Total Phenolic content

Fig-2 shows the bar graph illustrating the total phenolic content in different varieties of Capsicum annuum, measured as gallic acid equivalent (mg/g of extracted compound).

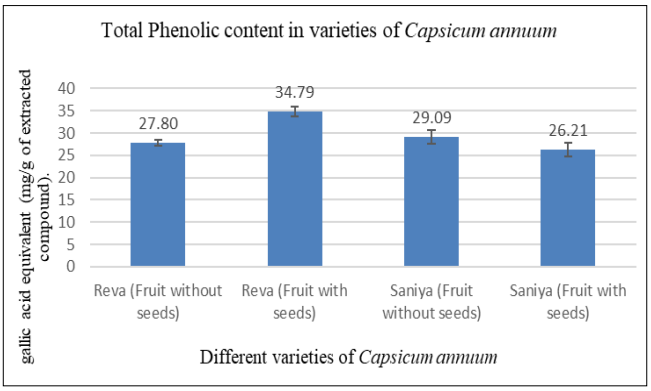

Fig 2: Shows the total phenolic contents of *Capsicum annuum* L. cultivar Reva and *Capsicum annuum* L. cultivar Saniya respectively

Reva (with seeds) recorded the highest phenolic content at 34.79 mg GAE/g, followed by Saniya (without seeds) at 29.09 mg GAE/g. Reva (without seeds) showed a phenolic content of 27.80 mg GAE/g, while Saniya (with seeds) recorded the lowest value at 26.21 mg GAE/g. In contrast to flavonoid trends, Reva fruits with seeds contained

significantly more phenolics than those without seeds, suggesting that seeds may contribute positively to phenolic content, particularly in the Reva variety. For the Saniya variety, the difference between fruits with and without seeds is less pronounced, but seeds still appear to slightly dilute phenolic concentration. Overall, Reva outperformed Saniya in phenolic accumulation, especially when seeds were included. The small error bars indicate minimal variation among replicates, suggesting consistent results. Thus, the phenolic content varies between varieties and between deseeded fruits and seeded fruits, with Reva seed showing the highest levels, implying potential for greater antioxidant activity.

Discussion

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993) [13]. All samples demonstrated a strong presence of carbohydrates, as indicated by the Molisch, Benedict, and Fehling's tests. This is consistent with the nutritional role of chili peppers as energy-rich foods. However, none of the samples tested positive for starch via the Iodine Test, suggesting that the carbohydrates are primarily present in simpler, more readily digestible forms rather than complex polysaccharides. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et. al., 2007) [12]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et. al., 2007) [6]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown & Rice-Evans, 1998; Krings & Berger, 2001) [4, 8]. Tannins bind to proline rich protein and interfere with protein synthesis (Yadav & Agarwala, 2011) [16]. In the present study, Saniya deseeded fruit exhibited the highest concentration of tannins and phenols, highlighting its potential as a strong antioxidant source. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro* (Yadav & Agarwala, 2011) [16]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996) [9]. Flavonoids are powerful antioxidants that help neutralize free radicals and oxidative stress, protecting cells from damage and aging. They also possess antimicrobial properties as well as UV protection ability helping in plant defense. Reva seeded fruits showed the highest concentration suggesting it may have strong anti-inflammatory and cardioprotective properties.

Conclusion

In conclusion, the comparative phytochemical analysis of *Capsicum annuum* L. cultivars Reva and Saniya from Gujarat revealed notable differences in their bioactive compound profiles, highlighting their unique nutritional and medicinal potentials. Both varieties exhibited strong carbohydrate presence, but Saniya deseeded fruits stood out with the highest levels of phenols, tannins, and saponins, suggesting potent antioxidant and therapeutic properties. Reva seeded fruits, on the other hand, showed a remarkable concentration of flavonoids indicating potential pharmacological value. Although terpenoids were absent across all samples, the varying presence of glucosides, steroids, and other compounds underlines the diversity in phytochemical composition between deseeded and seeded fruits, as well as between the two cultivars. These findings not only affirm the health benefits of chili peppers but also open avenues for further research into cultivar-specific medicinal applications.

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