ESTIMATION OF CAPSAICIN FROM SOME LOCAL VARIETY OF CHILLI (*CAPSI*
ANNUM L.) FROM TRIBAL AREA OF KALSUBAI HARISHCHANDRA GAD SANCTUAR

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Abstract

Chilli (Capsicum annum L.) is belonging to the family Solanaceae well known spices commonly used India. It is a vital commercial crop, cultivated for vegetable, spice, and value-added processed products. Chi one of the highest export spice from India and it is well known for its pungent taste. The Pungent teste of chilli is to Presence of capsaicin. The tribes residing in Kalsubai-Harishchandra gad Sanctuary area has conserve many varieties of the crop plants through their practices. These tribes cultivating chilli in Kitchen Garden since long t period for the daily use in food.

The objective of the work was to estimate capsaicin content in some locally collected chilli germpla from the Kitchen gardens of tribals residing Kalsubai Harishchandra gad Sanctuary Area in order to study a calculate percentage of Capsaicin from the local chilli verities. This parent species may be useful for further breed program.

Keywords: *Chilli (Capsicum annum L.), Kalsubai-Harishchandra gad Sanctuary, Capsaicin*

Introduction:

Chilli (*Capsicum annum* L.) fruit has been known all over the world as a hot and spicy with a characteris smell and taste. It is an economically important spices crop in India. This spice is belonging to the fam Solanaceae, originated from South and Central America where it is still under cultivation (Pickers 1997). The chilli can be distinguished by its pungency which varies with cultivar but generally higher smaller fruit types than larger thick-fleshed types.

Total production of chilli in India in the year 2021-22 was about 2092153 tonnes and Export was 601 tonnes. (Malhotra S, 2001) Chilli is a vital commercial crop, cultivated for vegetable, spice, and val added processed products (Kumar and Rai, 2005) and is an important constituent of many foods, addi flavour, vitamins A and C and pungency and is, therefore, indispensable to world food industries. Pungent teste of chilli is due to Presence of capsaicin. The Capsaicin is present in the Placental tis which hold the seeds of the fruit.

This was discovered and isolated in 1846 and then determine its structure in the year 1919 (Mark H Peter C, 2008). Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a naturally occurring alka

extracted from fruit of the Chilli. Capsaicin is a fat soluble, odourless, pungent tasting, off-white solid with a melting point of 62–65° C and a molecular weight of 305.4 k. Da. As it is not water soluble, alcohols and other organic solvents are used to solubilise capsaicin in topical preparations (Rains C and Bryson H, 1995). The temperature at which chili are grown, the position of the fruit on the plant, age of the plant and light intensity are all factors affecting the total amount of capsaicin in a given chili variety.

Moreover, environmental and nutritional conditions occurring during the cultivation of chilli can affect the capsaicinoid content. different types of chilli germplasm distributed throughout the country, it is necessary to collect and characterize them biochemically with a view to develop new variety. The objective of the work was to estimate capsaicin content in some locally collected chilli germplasm from the Kitchen gardens of tribals residing Kalsubai Harishchandra gad Sanctuary Area in order to study and calculate percentage of Capsaicin from the local chilli verities. This parent species may be useful for further breeding program.

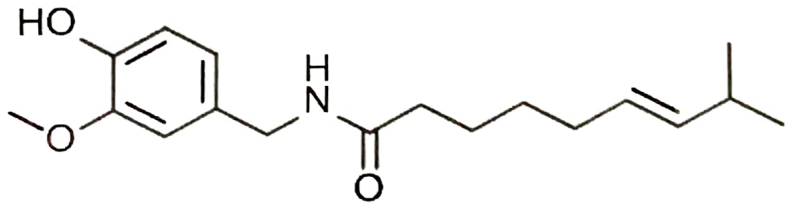


Figure 1: Molecular structure of capsaicin

Plant Morphology:

Capsicum annuum is a species of the plant genus Capsicum (peppers) native to southern North America and northern South America. This species is the most common and extensively cultivated of the five domesticated capsicums. The species encompasses a wide variety of shapes and sizes of peppers, both mild and hot, such as bell peppers, jalapeños, and cayenne peppers. Cultivars are descended from the wild American bird pepper still found in warmer regions of the Americas.

In the past some woody forms of this species have been called C. frutescens, but the features that were used to distinguish those forms appear in many populations of C. annuum and it is not a consistently recognizable feature in C. frutescens species. Moreover, crosses between C. annuum and C. frutescens aren't likely because seeds obtained from pollination between those two species (if the embryo survives) will not germinate.

Characteristics:

Although the species name annuum means "annual" (from the Latin annus "year"), the plant is not an annual but is frost tender. In the absence of winter frosts it can survive several seasons and grow into a large, shrubby perennial herb. The single flowers are an off-white (sometimes purplish) colour while the stem is densely branched and up to 60 cm (24 in) tall. The fruit are berries that may be green, yellow, orange or red when ripe. While the species can tolerate most frost-fee climates, C. annuum is especially productive in warm and dry climates.

Material and Method:

A. **Sample Collection**: Sample was collected from Kitchen gardens of tribal peoples residing forest area. The place from where sample was collected are (a) Bhandardara MND 311 (b) Kumshet MND 321 (c) Ghatghar MND 331 (d) Pendshet MND 341 and (e) Ratanwadi MN 351. samples were collected between September 2022 to October 2022. These samples we selected randomly. The collected samples were sun dried for one month then the samples we maintained at 4^0 C in the laboratory, where all samples were ground into powder and stored plastic bags at 4^0C for further analysis.

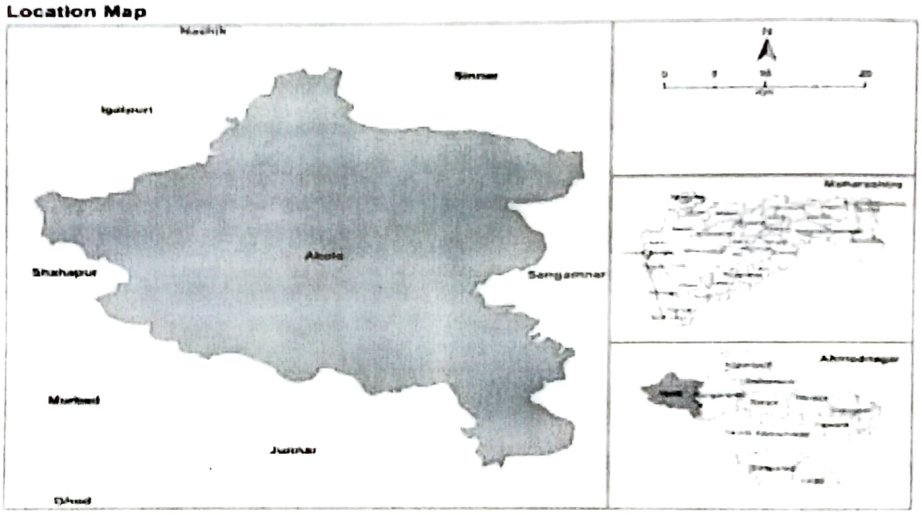


Figure 2: Location map

Method for measuring Capsaicin in chillies: (ASTA ANALYTICAL MANUAL METHOD N 21.3)

1. Apparatus

a) Standard flasks, 50 mL,100 mL & 200 mL capacity
b) Heating mantle of 500 mL capacity, with regulator
c) HPLC system with accessories as mentioned under Instrument conditions
d) Micro litre syringe capable of injecting 1 - 20µL.
e) Pipette, 10 mL
f) Balance, readable to 0.01 g.
g) Glass beads.
h) Boiling flask, 500 mL.
i) Whatman No. 1 filter paper (90 mm)/ syringe filter 0.45 µm.
j) Sample powdering mill or equivalent
k) Water cooled condenser.

2. Reagents:

a) Rectified spirit (chromatographically pure): Whenever fresh batch is purchased check for puri by gas chromatography and if necessary, distil the same before use for analysis.

b) Acetonitrile HPLC grade
c) Water HPLC grade
d) Acetic acid HPLC grade.
e) Acetone -HPLC grade.
f) Standard - N - Vanillyl - n - nonanamide, 97% (Sigma or equivalent).

Standard stock solution –

Weigh accurately 0.1g of the above standard and dissolve and make upto 100 mL with rectified spirit. Keep this solution as stock solution (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration.

Working standard 100 ppm

From the stock solution pipette 10 mL to the 100 mL standard flask and make up to the mark with rectified spirit. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.

Procedure:

Sample preparation: Whole chillies: After mixing & quartering, powder 100 g of the sample and pass Through the sieve ASTM No. 20. (850 om).

Entire sample: -

1. Weigh accurately 25.0 g of the above sample in duplicate into 500 mL boiling flask.
2. Add 200 mL rectified spirit and then several glass beads to aid boiling.
3. Reflux gently for 5 hours.
4. Allow to cool and then filter 3 to 4 mL through whatman no.1 filter paper pre- wetted with rectified spirit or 0.45µm syringe filter into stoppered test tubes.

Oleoresins: Accurately weigh 1 to 2 g oleoresin (increase sample size if total Cpsaicinoid concentration is below 1%) into 50 mL volumetric flask, being sure not to allow any oleoresin to coat the sides of the flask. Add 5 mL of acetone to flask and swirl acetone until sample is completely dispersed as evidenced by observing no oleoresin coating bottom of flask with neck at 45° angle. Slowly add rectified spirit, mixing completely by swirling with acetone while adding. Continue adding and mixing until solution becomes cloudy. Dilute to volume and mix well. Filter 3 to 4 mL through Whatman no.1 filter paper pre-wetted with rectified spirit or 0.45 µm syringe filter into stoppered test tubes.

Instrument condition:

HPLC System: Water TM 600 system controller Waters TM 486 tunable absorbance detector

- Waters 746 Data module / Waters millennum 32 software
- Waters U6K injector / Rheodyne injector
- Waters 717 plus Autosampler.

HPLC Column - 5 µm Waters Symmetry TM C18 (4.6x250 mm) steel column

Note: Use either degassed eluent or sparge with Helium before run. All solvent should be HPLC grade. Eluent used - Freshly prepared 60 % acetonitrile + 40 % water with 1 % acetic acid at 0.8 mL / minute flow.

Absorbance - 280 nm

Volume for injection - 5 to 10 µl

1. Switch on the instrument.
2. Wait for system configuration.
3. Press the setup function key to display the pump set up screen.
4. Enable sparging if necessary (appropriate reservoirs A B C D)
5. Press the direct function key and sparge helium at 100 mL/min for first 20 mts then reduce t to 30 mL / min for acetonitrile + water system. (If helium is used for sparging
6. Vent the eluent by keeping the small glass bottle under the open vent tubing (injection port) to on the vented eluent.
7. Turn the load/ inject handle to the inject (left) position and then the sample loading plug han to the open (vertical) position to open the vent port.
8. From the direct function key at the % A field, type a value of 100 to set the flow to 100 % eluent reservoir A.
9. Increase the flow by 0.1 mL / min upto 0.8 mL / min.
10. Attach the priming syringe, open the eluent drain - off valve (turn the knurled knob coun clockwise) and draw off 10 mL of eluent.
11. Repeat the above step, if air bubble is trapped in the delivery system (tube).
12. Close the eluent draw - off valve and remove the primary syringe keep an empty bottle at eluent outlet waste tube.
13. Keep the flow of eluent (0.8 mL / min) through the HPLC column.
14. When the pressure indicated in direct control screen becomes steady, the system is ready injection.
15. Load the sample (5 to 10 µl) into waters U6K manual injector or rheodyne injector (with 5 or µL sample loops), using micro litre syringe after rinsing in pure rectified spirit not less than times.
16. Turn the load / inject handle to the load (right) position and turn the sample loading plug han to the open (vertical) position.
17. Remove the sample loading plug.
18. Insert the syringe with sample / standard into the sample loading port until the syringe bott and empty the syringe file the loop.
19. Remove the syringe, replace the sample loading plug and turn the sample loading plug handl the closed (horizontal) position.
20. Chromatograms are stored in respective BINS of data module.
21. Make a file with run time parameters.
22. Reprocess the chromatograms of samples and take the report. (Calculation is made based on of standard capsaicin)
23. Run the system in pure acetonitrile at least half an hour before switching off the system. Alw prime the system when eluent is changed.

24. Switch off the detector, system controller at the unit and then main power of the above. Keep the plastic plunger to the eluent draw off valve.
25. Switch off the data module at the main power.
26. Discard the eluent from the waste bottle.
27. Change the prepared eluent from the.
28. reservoirs only when the system is used next time.

Autosampler: -The autosampler is switched on and the sample extracts taken in the vials are loaded in the carousel. In the Millenium32 software, the system is selected and the functions to be carried out are entered in samples set of the run mode.

Calculations:

a) Scoville Heat Units (SHU) are the sum of SHU of three major capsaicin.
Calculate SHU as follows:
Capsaicin, SHU = (C/A) x (Cs/WX) x (HC/RC).
Where: A = average peak area of standard; C = average peak areas for respective capsaicinoids from duplicate injections; Cs = concentration of std in mg/mL; WX = wt of sample in mg/mL; HC= heat factors for respective capsaicinoids; RC = response factors of respective capsaicinoids relative to standard.

b) Accepted heat factors and response factors: Capsaicin HC = 16.0 E + 06; RC = 0.89

c) Relative retention times: Capsaicin 1.00

d) Capsaicin content in percentage is calculated as follows:

$$\text{Capsaicin content (\%)} = \frac{\text{Total SHU}}{16 \times 10000}$$

Result and Reporting:

Result is given in SHU or in percentage as requested in laboratory information sheet. During the reporting in scoville heat unit correct the value to the nearest hundred and for percentage, correct the value to 2 decimals.

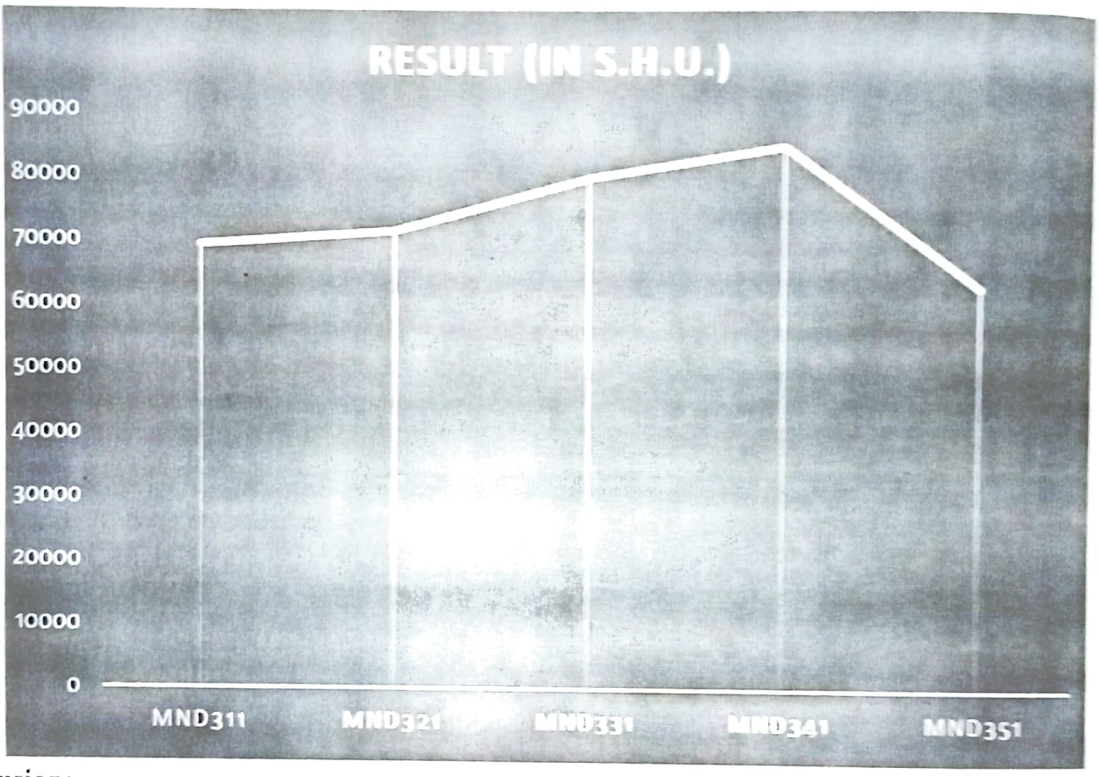
Table 1: Content of Capsaicin in scoville heat unit (S.H.U.)

Sr. No.	Samples Number	Location of Samples	Result (In S.H.U.)
1.	MND311	Bhandardara	69408
2.	MND321	Kumshet	71548
3.	MND331	Ghatghar	79845
4.	MND341	Pendshet	84675
5.	MND351	Ratanwadi	62857

Where, The Sample numbers 1st latter denoted Tour Number, second number denote Location and third number denote Sample species

Results and Discussion:

The collected samples from field area were shown highly pungent to moderately pungent Whereas the sample collected from Pendshet MND 341 and Ghatghar MND 331 are highly pungent wh the sample from Bhandardara MND 311, Kumshet MND 321 and Ratnwadi MND 351 are Moderate pungent.



Conclusion:

The Chilli (*Capsicum annum* L.) samples were collected from the field were tested for th Percentage of Capsaicin and the Sample number MND341 is found high percentage of Capsaicin and can be utilized for pharmaceutical use Sample number. MND 351 has very low percentage of Capsaici which is at moderate percentage as per standard.

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