RESEARCH PAPER

Phytochemical Analysis, In-Vitro Antioxidant, and Anti-Ulcer Activity of Bhut Jolokia (*Capsicum chinense* Jacq.) Ethanolic Extract

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Abstract

Background & Objective: *Capsicum chinense* (Bhut Jolokia / King Chilli) is renowned for its intense pungency and medicinal properties. This study evaluates the physicochemical properties, phytochemical composition, *in-vitro* antiulcer potential, and antioxidant profile of *C. chinense* extracts collected from Sonapur, Kamrup (M) district, Assam, India.

Methods: Botanical authentication was confirmed at Gauhati University (Accession No. GUBH20027). Crude fruit samples were extracted using ethanol and water to evaluate total ash value, solvent extractive yields, and qualitative phytochemical profiles across major secondary metabolite classes. The *in-vitro* antiulcer capacity was assessed by measuring the Acid Neutralizing Capacity (ANC) at concentrations ranging from 100 to 1500 mg against standard Al(OH)3 + Mg(OH)2 500mg. Antioxidant capacity was evaluated via the DPPH radical scavenging assay, alongside total phenolic content (TPC) and total flavonoid content (TFC) estimations using gallic acid and quercetin/catechin calibration curves, respectively.

Results: Physicochemical analysis revealed a total ash value of 2.11%, with water-soluble and alcohol-soluble extractive values of 25.6 % w/w and 22.4 % w/w, respectively. Qualitative screening confirmed the presence of alkaloids, carbohydrates (ketoses), reducing sugars, proteins/amino acids, flavonoids, and phenolic compounds (including carotenoids), while cardiac glycosides and tannins were absent. The aqueous extract demonstrated potent, dose-dependent acid-neutralizing activity, displaying ANC values between 19.2 and 24.9 mEq/g, outperforming the standard antacid 14.7 mEq . The DPPH assay demonstrated significant free-radical scavenging activity with an IC50 value of 93.03 g/mL for the test extract compared to 64.10 mu g/mL for standard Butylated Hydroxyanisole (BHA). Calibration data corroborated substantial concentrations of total phenolics and flavonoids.

Conclusion: The findings demonstrate that (Bhut Jolokia)*Capsicum chinense* possesses strong natural acid-neutralizing and free-radical scavenging properties, driven by its rich phenolic and flavonoid profile. These results highlight its therapeutic potential as a natural candidate for managing gastric hyperacidity and oxidative stress-related disorders.

Keywords: *Capsicum chinense*, Bhut Jolokia, Phytochemical screening, Acid Neutralizing Capacity, Antiulcer, DPPH antioxidant assay, Phenolics

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INTRODUCTION:

One of the most extensively grown spice crops in the world, chilli peppers (Capsicum spp.), a member of the nightshade family Solanaceae, are prized for their culinary, commercial, and therapeutic uses [1-2] .There are many species in the genus Capsicum, but Capsicum chinense Jacq. is known to produce cultivars with the highest heat profiles [3-4] Jolokia, Bhut (syn. Ghost Pepper, also known as Naga Jolokia, is an interspecific cultivar of C. chinense that is native to Northeastern India, mostly in Assam, Nagaland, Manipur, and Arunachal Pradesh[5-6]

From 2007 until 2011, Guinness World Records declared Bhut Jolokia to be the hottest chili pepper in the world.Later, American chili types such as the Carolina Reaper claimed this distinction.Bhut Jolokia has a maximum heat level of 1,041,427 Scoville Heat Units, according to studies utilizing High-Performance Liquid Chromatography.

Bhut jolokia is mainly grown in North-eastern part - Arunachal Pradesh, Nagaland, Manipur and Assam of

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India. Bhut jolokia belongs to family Solanaceae which belongs to *Capsicum chinense* species[7]. Bhut jolokia held title of 'world's hottest pepper' from 2007 to 2011 until superseded by Carolina reaper of South Carolina, US[8]. The hotness of a chilli is measured in SHU (Scoville Heat Units) with Bhut jolokia obtained highest SHU of 1,041,427 reported by Frontal agritech in 2004 measured by HPLC analysis[9].

The hotness of a chilli is mainly due to presence of capsaicin [N-(4-hydroxy-3 methoxybenzyl)-8-methylnon-trans-6-enamide [10]. The capsaicin content in Bhut jolokia was reported to be 0.75 - 4.65 % of its dry weight[11].

Capsaicin content of Bhut jolokia is highly dependent on regional climatic factor as it has been reported to drop by 50% by growing Bhut jolokia pepper in Central India compared to North-eastern India[12]. Capsaicin readily shows change in biological activity specifically digestive, nervous system and cardiovascular system[13-14].

Traditionally, consuming chilli regularly in small quantities has been noted to help heal asthma and Gastro-intestinal abnormalities[15]. Muscle pain and tooth ache pain can be reduced significantly by applying hot infusion of chilli on affected area[16]. The high concentration of capsaicin extracted from Bhut jolokia is used in various pharmacological applications. Capsaicin is used as pain reliever by reducing pain and inflammatory heat from fibromyalgia patient[17]. Capsaicin showed anti-obesity effect by increase in lipid metabolism and thermogenesis by regular consumption[12]. Capsaicin can alter membrane fluidity in platelets thus inhibiting platelet aggregation and clotting factors VIII and IX in turn reducing chance of cardiovascular disorder[18-20]. Capsaicin has anticancer ability[11], capsaicin kills prostate cancer cells and stops migration of breast cancer cells [21-22]. Capsaicin also has anti-diabetic activity and decrease in blood glucose level was observed in KK-A^y[obese/diabetic] mice Capsaicin showed Hepatoprotective effects in rat induced with carbon tetrachloride liver injury[23].

MATERIALS AND METHOD:

Sample collection – The plant material was collected from the surroundings of Sonapur, Kamrup (M) district, Assam, India .Authenitication of plant the selected plant was donein Guwahati University, Accession no. GUBH20027, ASSAM (INDIA). sample was oven dried and stored in 8-12°C in dry condition.

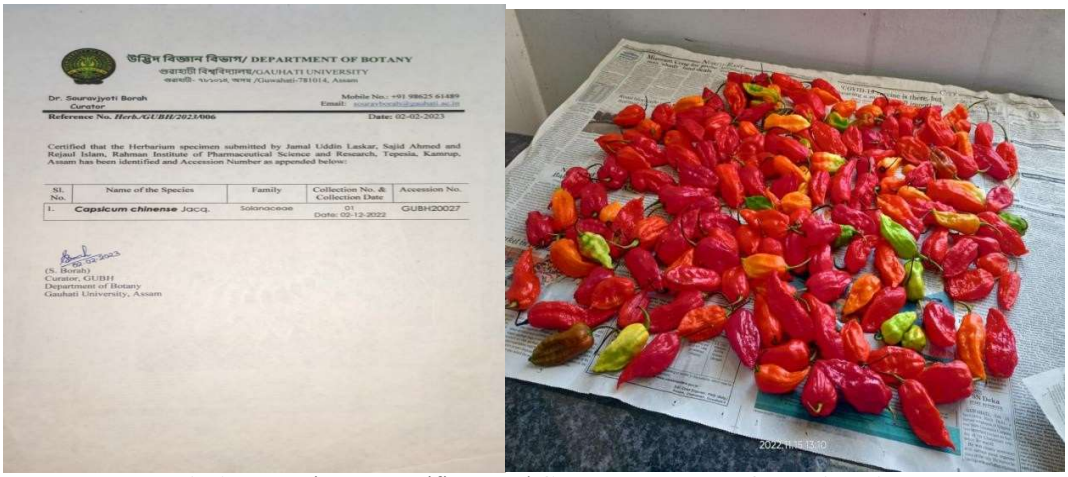

Fig 1: Accession no. certificate and **Collected samples of Bhut jolokia**

Preparation of extract:

Dried chilli pods weighing 10gm were taken and cut into very small pieces. The small cut pieces were extracted with 50ml of ethanol as solvent. The flask was sealed and kept for 3 days in cool, dry place. On 4th day 10ml of chilli-solvent was concentrated on hot plate for 1 minute which was later dried to be used as extract[24].

Phytochemical Analysis:

Chemical profile of the ethanolic Bhut jolokia pepper extract was prepared by performing alkaloid test, steroid test, Terpenoid test, Glycoside test, Saponin test, Tannin test, Flavonoid test from standard procedures [25].

In-vitro Evaluation of Antiulcer Activity:

Acid Neutralizing Capacity: The aqueous extract of acid-neutralizing capacity value are 100mg, 500mg, 1000mg, 1500mg. The aluminium hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl wasimmediately titrated until the pink colour is attained 15. The moles of acid neutralized is calculated by, Moles of acid

neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract[26]

Antioxidant assay:
DPPH radical scavenging assay was used to check for antioxidant activity. 500µl extract was added to 3ml methanol to form working sample. Different concentrations of samples were taken (control, 20µl - 120µl) in 7 test tubes and was made up to 1 ml with methanol added 1ml DPPH in all test tubes and incubated for 45 mins in dark environment. The test tubes were checked for OD at 517nm[27-29].

RESULTS AND DISCUSSIONS:
The result for phytochemical analysis for ethanolic Bhut jolokia pepper extract is detailed in Table 1. Presence of flavonoids confirmed the antioxidant activity in pepper[22, 23]. The presence of alkaloids, terpenoids, saponins, flavonoids in aqueous, acetone, acetonitrile, chloroform, butanol extract of Bhut jolokia pepper has been reported[24,25].

1. Phytochemical Screening of *Capsicum chinense* (Ethanolic Extract)

Table 1: Summary of Phytochemical Profile for *Capsicum chinense*

Category	Sl. No.	Chemical Test	Observation	Result
I. Alkaloids	1	Dragendorff's test	A reddish-brown precipitate	**Positive**
	2	Mayer's test	A creamy white/yellow precipitate	**Positive**
	3	Picric acid test	An orange coloured solution	**Positive**
	4	Tannic acid test	A buff colour precipitate	**Negative**
Conclusion:		**Alkaloids are PRESENT**		
II. Carbohydrates	1	Resorcinol test	A rose colour {ketones}	**Negative**
	2	Test for pentoses	A red colour	**Negative**
	3	Test for starch	A canary colouration	**Negative**
	4	Seliwanoff's Test	A rose red colour {ketoses}	**Positive**
Conclusion:		**Carbohydrates are PRESENT**		
III. Reducing Sugars	1	Benedict's test	Green/yellow/red colour	**Positive**
	2	Fehling's test	A red precipitate	**Positive**
	3	Borntrager's test	A pink coloured solution	**Negative**
	4	Modified Borntrager's test	A rose-pink to blood red coloured solution	**Positive**
	5	Aqueous NaOH test	A yellow colour	**Positive**
	6	Concentrate H2SO4 test	A brown ring	**Positive**
Conclusion:		**Reducing sugars are PRESENT**		
IV. Cardiac Glycosides	1	Keller-Kiliani test	A blue coloured solution (in acetic acid layer)	**Negative**
	2	Test for Cardenolides	A red colour, fades to brownish yellow	**Positive**
	3	Bromine water test	A yellow precipitate	**Negative**
	4	Baljet test	A yellow-orange colour	**Negative**
Conclusion:		**Cardiac Glycosides are ABSENT**		
V. Proteins & Amino Acids	1	Millon's test	A white precipitate	**Positive**
	2	Ninhydrin test	A purple coloured solution {Amino acids}	**Negative**
	3	Xanthoproteic test	A yellow coloured solution	**Positive**
Conclusion:		**Proteins and Amino acids are PRESENT**		
VI. Flavonoids	1	Ferric chloride test	A green precipitate	**Negative**
	2	Zinc-hydrochloride reduction test	A white precipitate	**Positive**
	3	Ammonia test	A purple coloured solution {Amino acids}	**Positive**
	4	Conc. H2SO4 test	A white precipitate	**Positive**
Conclusion:		**Flavonoids are PRESENT**		
VII. Phenolic Compounds	1	Iodine test	A transient red colour	**Positive**

	2	Ferric chloride test	Dark green/bluish black colour	**Negative**
	3	Lead acetate test	A white precipitate	**Positive**
	4	Test for Carotenoids	A blue colour at the interface	**Positive**
Conclusion:		**Phenolic compounds are PRESENT**		
VIII. Tannins	1	Braymer's test	Blue-green colour	**Negative**
	2	10% NaOH test	Formation of emulsion {Hydrolysable tannins}	**Negative**
	3	Lead sub acetate test	A creamy gelatinous precipitate	**Positive**
Conclusion:		**Tannins are ABSENT**		

Based on experimental results, the water extract contains:

- **Present:** Alkaloids, Carbohydrates (specifically ketoses), Reducing Sugars, Proteins/Amino Acids, Flavonoids, and Phenolic compounds (including carotenoids).
- **Absent:** Cardiac Glycosides and Tannins.

1.1. Determination of Ash Value

Parameter	Observed Value
Weight of empty silica crucible (w)	18.78 g
Weight of crucible + sample (w1)	20.79 g
Weight of crucible + ash (w2)	19.22 g
Weight of ash (w2 - w)	0.44 g
Calculated Ash Value (as per text)	**2.11%**

Table 2: Determination of Ash Value

1.2. Extractive Values

*Weight of powdered crude drug used = **2.5 g** (extracted into 50 mL solvent, with 25 mL used for drying).*

Extractive Type	Empty Dish Weight (g)	Dish + Dried Extract Weight (g)	Weight of Dried Extract in 25 mL (g)	Extractive Value (% w/w)
Water Soluble	93.95	94.27	0.32	**25.6%**
Alcohol Soluble	100.98	101.26	0.28	**22.4%**

Table 3: Extractive Values

Summary of Results for King Chilli

- **Total Ash Value:** 2.11%
- **Water-Soluble Extractive Value:** 25.6% w/w
- **Alcohol-Soluble Extractive Value:** 22.4% w/w

2. DETERMINATION ANTIULCER ACTIVITY:

2.1. Acid Neutralizing Capacity

The in-vitro acid-neutralizing capacity (ANC) of the aqueous extract of *Bhut Jolokia* (King Chilli) was evaluated at four distinct concentrations (100 mg, 500 mg, 1000 mg, and 1500 mg). A combination of Aluminium Hydroxide and Magnesium Hydroxide [{Al(OH)}_3 +{Mg(OH)}_2] at a concentration of 500 mg was utilized as the standard reference drug. The findings indicate that the aqueous extract possesses an efficient, concentration-dependent acid-neutralizing profile. The ANC values per gram for the extract concentrations at 100 mg, 500 mg, 1000 mg, and 1500 mg were determined to be 24.9, 23.1, 22.6, and 19.2 mEq/g respectively, compared to 14.7 mEq/g for the standard antacid formulation. The complete analytical data is compiled in Table 5.

Test Material	Concentration (mg)	Volume of NaOH Consumed (mL)	mEq of Acid Consumed	ANC per gram of Antacid (mEq/g)

Aqueous Extract	100	41.2	10.00	24.9
Aqueous Extract	500	39.5	12.25	23.1
Aqueous Extract	1000	42.0	9.50	22.6
Aqueous Extract	1500	36.5	13.00	19.2
Standard [Al(OH)3+Mg(OH)2]	500	45.6	7.55	14.7

Table 5: In-Vitro Acid Neutralizing Capacity (ANC) Profile

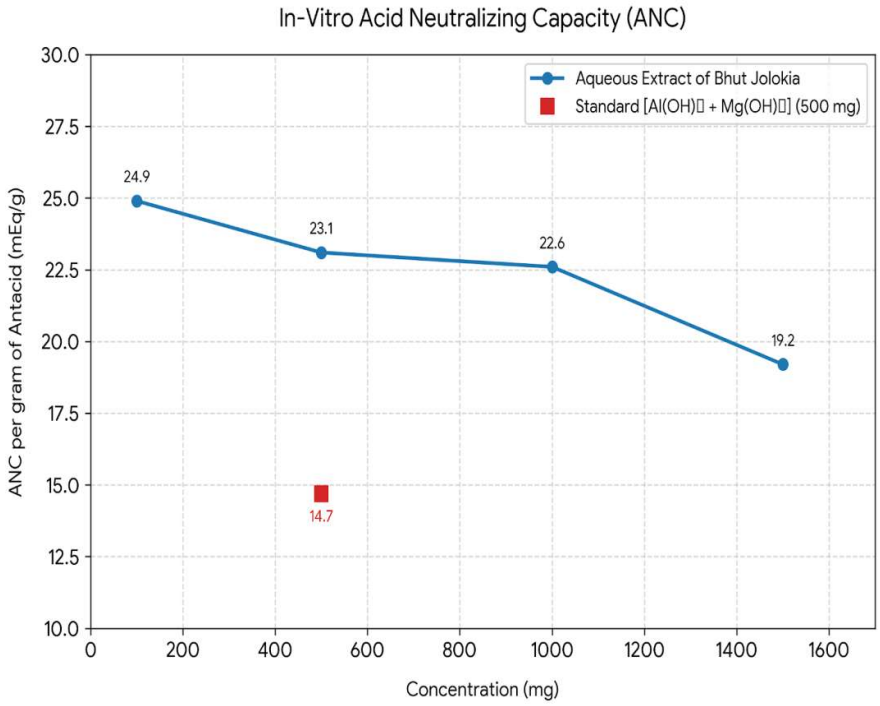

Fig2: Graphical representation of In-Vitro Acid Neutralizing Capacity

Discussion

The experimental results demonstrate that the aqueous extract of *Bhut Jolokia* possesses a strong, inherent acid-neutralizing capacity. When evaluating the total capacity relative to the sample mass, the extract at all tested concentrations exhibited a higher ANC value per gram than the standard commercial antacid drug [{Al(OH)}_3 + {Mg(OH)}2] (14.7 mEq/g). The capacity profile demonstrates that smaller sample masses exhibit a highly efficient neutralizing capacity per unit gram, validating the potential of the crude drug extract as an effective natural antacid agent.

3. DETERMINATION ANTOXIDANT ACTIVITY:

3.1. Antioxidant assay:

Result of Antioxidant assay by DPPH oxygen scavenging assay of ethanolic Bhut jolokia pepper extract has been presented in Table 2.

DPPH free radical scavenging activity percentage was measured by equation[28]:

$$\text{Inhibition percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

S. No.	Concentration (µg/mL)	% Inhibition (BHA Standard)	% Inhibition (Aqueous Test Extract)	IC50 Standard (µg/mL)	IC50 Test Extract (µg/mL)
1	10	22.95	10.23	**64.10**	**93.03**
2	20	34.24	21.11	—	—
3	40	48.66	35.61	—	—

4	60	62.29	41.21	—	—
5	80	70.24	54.32	—	—
6	**100**	**82.73**	**62.54**	**—**	**—**

Table 6: DPPH Radical Scavenging Activity (% Inhibition) and IC 50 Values

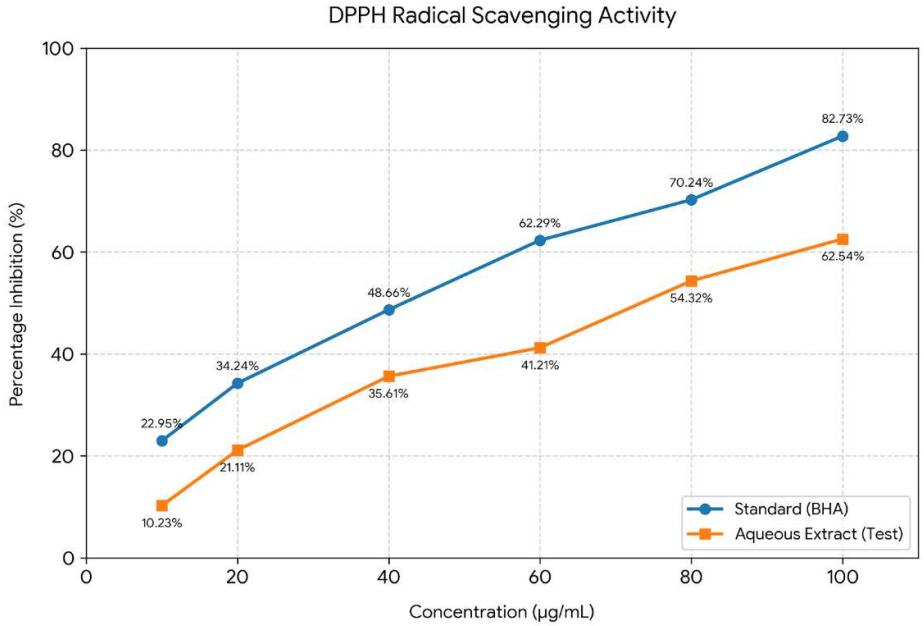

Fig-3: DPPH Radical Scavenging Activities of Aqueous Extract and Butylated Hydroxyanisole (BHA)

3.2. TOTAL PHENOLICS CONTENT

Total phenolic content was estimated by gallic acid (Fig-6) and expressed as mg gallic acid equivalent (GAE)/g of extract. Table-7 represents the analytical data for phenolics content of the aqueous extract of *Capsicum chinense*. Data clearly show a considerable amount of total phenolic content in aqueous extract of *Capsicum chinense.*

S. No.	Concentration (µg/mL)	Absorbance (765 nm)
1	20	0.11
2	40	0.14
3	60	0.24
4	80	0.29
5	100	0.38

Table 7: Absorbance of Gallic Acid for Standard Calibration Curve lambda max = 765nm

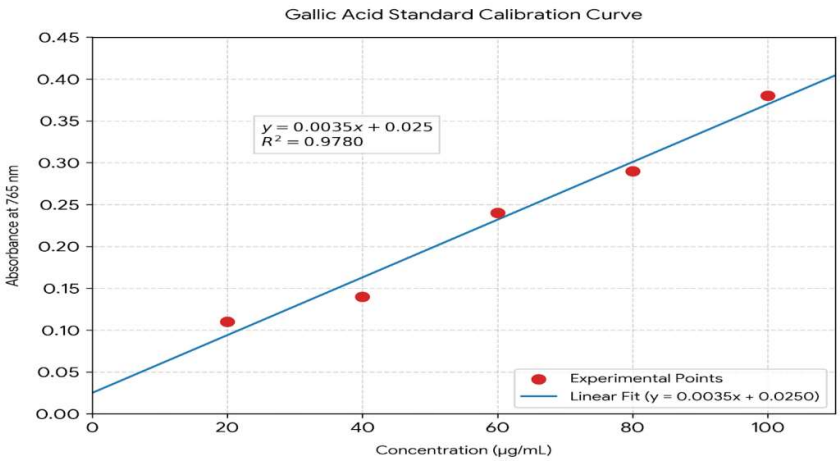

Fig-5: Standard Calibration Curve of Gallic Acid for TPC Determination

3.4. TOTAL FLAVONOIDS CONTENT:

Total flavonoids content of aqueous extract of *Capsicum chinense* was expressed as mg Catechin equivalents/ g of extract (Fig-7). Samples were analyzed in triplicate. Table 8 represents the analytical data for flavonoid content of the aqueous extract of *Capsicum chinense*.

S. No.	Concentration (µg/mL)	Absorbance (765 nm)
1	10	0.023
2	20	0.042
3	40	0.087
4	60	0.114
5	80	0.159
6	100	0.211

Table 8:The analytical data for flavonoid content of the aqueous extract of *Capsicum chinense*.

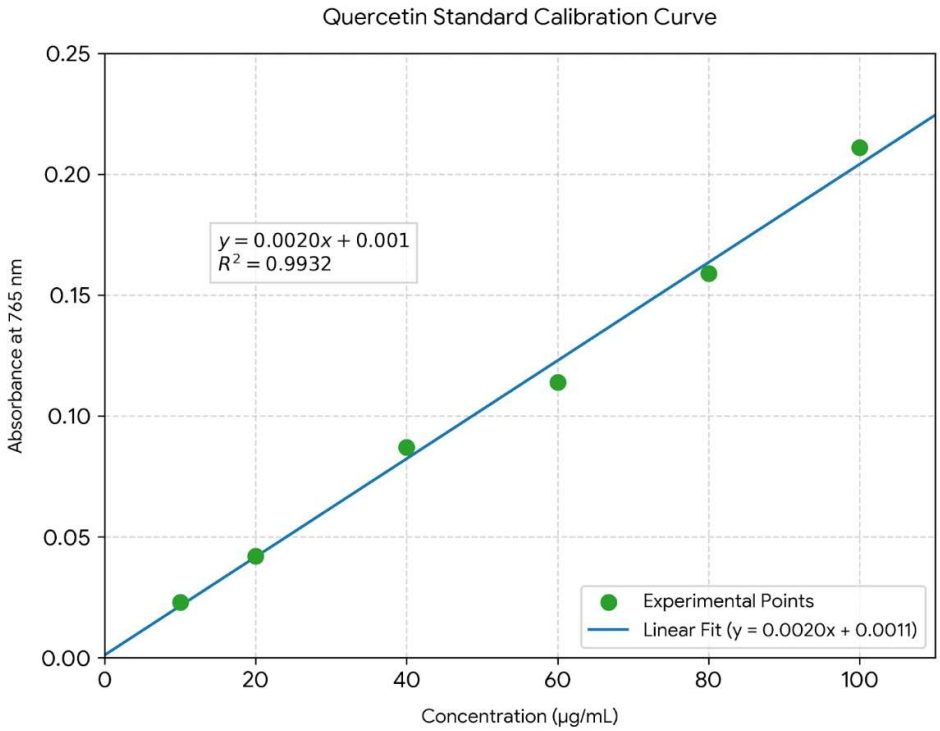

Fig-6: Standard Calibration Curve of Quercetin for TFC Determination

CONCLUSION:

The ethanolic extract preparation is simple and easier to prepare that other solvent extraction. From phytochemical Alkaloids, Carbohydrates (specifically ketoses), Reducing Sugars, Proteins/Amino Acids, Flavonoids, and Phenolic compounds (including carotenoids) are confirmed to present and Cardiac Glycosides and Tannins are absent. The experimental results demonstrate that the aqueous extract of *Bhut Jolokia* possesses a strong, inherent acid-neutralizing capacity. When evaluating the total capacity relative to the sample mass, the extract at all tested concentrations exhibited a higher ANC value per gram than the standard commercial antacid drug [{Al(OH)}_3 + Mg(OH)}2] (14.7 mEq/g). The capacity profile demonstrates that smaller sample masses exhibit a highly efficient neutralizing capacity per unit gram, validating the potential of the crude drug extract as an effective natural antacid agent. From antioxidant analysis, ethanolic Bhut jolokia extract shows good antioxidant activity at low concentration of 20µl.

CONFLICTS OF INTEREST:

The authors declare that they do not have any conflict of interest.

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