Phytochemical analysis, Antimicrobial and Antioxidant assay of Bhut Jolokia pepper

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ABSTRACT:

The presented work represents phytochemical analysis, Antioxidant assay and Antimicrobial activity of Bhut jolokia pepper (*Capsicum chinense Jacq)* extracted by ethanol as solvent. The ethanolic extract of Bhut jolokia pepper showed the presence of terpenoids, steroids, saponins and flavonoids. Antimicrobial assay was done with varying concentration (250-1000µg/ml) of pepper extract using tetracycline as control by well diffusion method, the extract at 750µg/ml shown best inhibition zone and *Staphylococcus aureus* showed the highest zone of inhibition at all concentration compared to other bacteria species with maximum zone of inhibition of 27mm. The DPPH scavenging assay for antioxidant activity at 517nm showed positive activity for scavenging, scavenging increased with the concentration of extract. Thus, Bhut jolokia could be effective in treating diseases caused by *Staphylococcus aureus* from antimicrobial assay result.

KEYWORDS: Bhut jolokia, phytochemical analysis, DPPH scavenging assay, Antimicrobial assay, *Staphylococcus aureus.*

INTRODUCTION:

Bhut jolokia is grown in North-eastern part - Arunachal Pradesh, Nagaland, Manipur and Assam of India. Bhut jolokia belongs to family Solanaceae which belongs to *Capsicum chinense* species[1]. Bhut jolokia held title of 'world's hottest pepper' from 2007 to 2011 until superseded by Carolina reaper of South Carolina, US[2]. 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[3].

The hotness of a chilli is mainly due to presence of capsaicin [N-(4-hydroxy-3 methoxybenzyl)-8-methylnon-trans-6-enamide][4]. The capsaicin content in Bhut jolokia was reported to be 0.75 - 4.65 % of its dry weight[5].

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[6]. Capsaicin readily shows change in biological activity specifically digestive, nervous system and cardiovascular system[7,8].

Traditionally, consuming chilli regularly in small quantities has been noted to help heal asthma and Gastro-intestinal abnormalities[9]. Muscle pain and tooth ache pain can be reduced significantly by applying hot infusion of chilli on affected area[10]. 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[11]. 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[11,13]. Capsaicin has anticancer ability[11], capsaicin kills prostate cancer cells and stops migration of breast cancer cells[14,15]. Capsaicin also has anti-diabetic activity and decrease in blood glucose level was observed in KK-A^y[obese/diabetic] mice[16]. Capsaicin showed Hepatoprotective effects in rat induced with carbon tetrachloride liver injury[17].

MATERIALS AND METHOD:

Sample collection – The chilli sample was bought from local market in and around Chennai and authenticated by Dr. K. Rajagopal, Botanist, Ramakrishna Mission Vivekananda College of Arts and Science, Chennai. Chilli sample was oven dried and stored in 8-12°C in dry condition. The bacteria sample – *Staphylococcus aureus* (MTCC 737), *Shigella flexneri* (MTCC 1457), *Micrococcus luteus* (MTCC 11948), *Bacillus subtilis* (MTCC 441), *Proteus vulgaris* (MTCC 744) were obtained from MTCC.

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[18].

Fig 1: Ethanol extract of Bhut jolokia pepper extract

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[19].

Antimicrobial assay:

Well diffusion method was performed to obtain zone of inhibition for antimicrobial activity. 0.01ml of each human pathogenic bacteria [*Staphylococcus aureus, Shigella flexneri, Micrococcus luteus, bacillus subtilis, Proteus vulgaris*] were spread on agar plate with different concentration of sample [250µg/ml, 500µg/ml, 750µg/ml, and 1000µg/ml] and control as tetracycline 20 µl concentration was used for each plate. The mentioned concentrations of sample with control was loaded in well and were incubated for 24 hours at 37°C[20].

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[21].

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].

Table1: Chemical profile of ethanolic extract of Bhut jolokia

S. No.	Phytochemicals	Inference
1	Alkaloids	Positive
2	Terpenoids	Positive
3	Glycosides	Negative
4	Steroids	Positive
5	Saponins	Positive
6	Tannin	Negative
7	Flavonoids	Positive

Result of Antioxidant assay by DPPH oxygen scavenging assay of ethanolic Bhut jolokia pepper extract has been presented in Table 2. A highest of 80% inhibition at 600µg of Bhut jolokia extract has been reported[26] and *Capsicum chinense* species has shown highest antioxidant activity in range of 60-80% than *C.annuum* and *C.frutescens*[27].

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

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

Table 2: Optical density at 517nnm against respective concentration

Concentration (µL)	Optical density at 517 nm
control	0.344
20	0.166
40	0.106
60	0.102
80	0.0961
100	0.082

Fig 2: DPPH radical scavenging activity of Bhut Jolokia pepper extract

The resultant zone of inhibition by well diffusion method for antimicrobial activity was found (Table 3). Best concentration for reasonable zone of inhibition in bacterial colonies compared to other concentration of sample used is at 750µg/ml, while *Staphylococcus aureus* showing highest zone of inhibition to all concentration of ethanolic Bhut jolokia pepper extract. The *S.aureus* species shows better zone of inhibition than other bacterial species tested at all concentration, higher *S.aureus* inhibition has also been reported by aqueous Bhut jolokia extract of 16mm at 50µg concentration[25] and zone of 21mm in similar species of *S.aureus*[24], a Bhut jolokia variety showed highest 12mm inhibition zone at 75% extract[29]. *M.luteus* has least zone of inhibition against Bhut jolokia extract of 15 mm while similarly low zone of inhibition has been reported of 18 mm at 500µg/ml[30]. *Aliivibrio fischeri* species showed 19 mm inhibition zone by acetonitrile extract of Bhut jolokia pepper[31]. Other bacterial species - *Salmonella typhimurium, Salmonella paratyphi, Klebisella pneumonia, Bacillus cereus, Vibrio cholera, Penicillium chrysogenum, Helicobacter pylori* showed good zone of inhibition by various Bhut jolokia extracts[8,9,24,25,30].

Fig 3: Comparison of zone of inhibition in spread plate culture

Table 3: Zone of inhibition on bacterial colony petri plate against range of concentration of sample extract

Organism	Zone of Inhibition (mm)	Concentration of sample (µg/ml)				
		250	500	750	1000	Control
S.aureus		18	20	27	29	11
S.flexneri		12	14	19	20	13
B.subtilis		12	18	22	27	11
M.luteus		10	12	14	15	19
P.vulgaris		15	18	22	22	11

Fig 4: Zone of inhibition for range of concentration of sample extract in *Staphylococcus aureus* colony petri plate.

Fig 5: Zone of inhibition for range of concentration of sample extract in *Micrococcus luteus* colony petri plate.

CONCLUSION:

The ethanolic extract preparation is simple and easier to prepare that other solvent extraction. From phytochemical analysis, presence of alkaloids, terpenoids, saponins, flavonoids, steroids are confirmed. From antioxidant analysis, ethanolic Bhut jolokia extract shows good antioxidant activity at low concentration of 20µl. From antimicrobial activity, *Staphylococcus aureu*s show good inhibition against Bhut jolokia at all range concentrations while at 750 µg/ml concentration has effective inhibition capability for all pathogens used. Further research will be to compare the ethanolic Bhut jolokia extract against various commercially available antibiotics with human pathogens in antimicrobial activity.

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CONFLICTS OF INTEREST:

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

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