



A Review on Recent Researches on *Bhut jolokia* and Pharmacological Activity of Capsaicin

Sangeeta Baruah*, Md K. Zaman, Plazapriya Rajbongshi, Simanti Das
Department of Pharmaceutical Sciences, Dibrugarh University-786004, Assam, India.
*Corresponding author's E-mail: sangeetabaruah03@gmail.com

Accepted on: 14-11-2013; Finalized on: 31-01-2014.

ABSTRACT

Bhut jolokia belongs to the Genus *Capsicum* and species *chinense*, which is World famous for its heat content. The plant is native to the Northeastern states of India and it is used traditionally in the treatment of various ailments besides using in the culinary dishes for flavour. The plant came in limelight when it was announced as the hottest chilli in the World in 2007. The plant has not been explored much regarding medicinal activity and very less literature information is available. This review includes the plant description, traditional uses, chemical constituents, pharmacological activity of capsaicin and recent researches.

Keywords: *Bhut jolokia*, Capsaicin, Traditional uses, Recent researches.

INTRODUCTION

Capsicum species are cultivated as vegetable and also they are used as condiment crops. They have been incorporated into a number of medicinal preparations in the ancient literature around the World.¹ Chilli peppers have been a part of the human diet in since 7500 BC. It has been found out in archaeological survey at sites located in South Western Ecuador that chilli peppers were domesticated more than 6000 years ago.² Portugese traders were the first to bring chilli peppers to Asia. Beside using chilli peppers in culinary dishes they posses medicinal properties which are used in the treatment of various diseases. Chilli peppers are widely used in India and in the Northeastern Indian states as traditional medicine for treatment of various ailments, as well use them to increase the taste and flavour of food.

The *Bhut jolokia* plant is wonderful gift of nature as its fruit is one of the hottest chilli in the World and at the same time it possess a pleasant and palatable aroma. The "*Bhut jolokia*" or "Ghost chilli" is second hottest chilli in the World. It is also known by other names such as "Naga Jolokia" (named after the ferocious Naga warriors of passed time), "Bih jolokia" (Bih means poison) and as ghost chilli pepper. "Naga king chilli" refers to the chilli's large pod sizewhich was published in the Asian Agri-History journal.³ It also known as Naga Morish, Raja Mirchi, Dorset naga, Nagahari (its derivative from Bangladesh). In Manipur, the chilli is called Umorok or Oo-morok.⁴ The two term 'Bih' or 'Bhut' are now used popularly in the scientific literature. In the recent years the traditional crop of Northeast India is gaining importance because it has been reported as one the hottest chilli in the World.

Plant description

Bhut jolokia mainly belongs to the species *Capsicum Chinense* Jaqç, Family: Solanaceae. The plant grows to a height of 57-129cm at 6 months.³ It may even grow taller

in semi-perennial situation. The leaves have a characteristic crinkle look as *C.chinense* species. Flowers are greenish white with a touch of light brown. The anthers are blue while the filaments are purple.³

Fruits are light green which turn bright red at maturity. The fruit posses 4-5 locules and bears about 25-35 slightly wrinkled seeds. There are at least three distinct colours found in *Bhut jolokia* like light red, dark red and orange.³ The following trends have been recorded such as:

- Young light green coloured fruits turn orange at maturity.³
- Young dark green coloured fruits turn dark red at maturity.³
- Young green coloured fruits turn light red at maturity.³
- Young dark green coloured fruits turn dark chocolate at maturity.³



Figure 1: Picture of *Bhut jolokia* fruit

Cultivation of *Bhut jolokia*

The chilli is widely cultivated in Northeastern states of India such as Assam, Nagaland, Manipur etc. In Northeast India there are two planting time, kharif and rabi. Kharif cultivation is mainly done in the hilly states of the region which starts in February - March and harvesting is done from May-June onwards. As a rabi crop it is grown in the plains of Assam during September-October. In North eastern state Nagaland the farmers practise Jhum cultivation (shifting cultivation) in paddy fields as sporadic with summer paddy and also in small homestead garden which can be kept for 2-3 years beyond which the fruit size reduces. In homestead traditional gardens, the farmers prefer to grow the crop in the shade rather in sunny places. In Jhum cultivation direct seeding is practiced in paddy fields and the peak harvest time is between August-September.³

Alternatively the cultivation can be done by growing seedling in seed bed and then transferring to main bed.³ The germination time is long which about 150-160 days and so the seeds can be treated with fungicides and insecticides to avoid damage. During the crop growth several diseases are seen such as fungal disease and slow wilting.³ The chilli pods are collected when they are bright red or orange. The crop can be grown under diverse soil and climatic condition from the fact that it is grown in few other parts of India and neighbouring countries like Bangladesh and Srilanka. It requires well drained sandy loam soil, clay loam or laterite soil.

Traditional uses of *Bhut jolokia*

The chilli variety is indigenous to the Northeast region of India and it is an important crop of the Northeast India but scientifically it has not been explored to its fullest. It has also been used conventionally in treating various human ailments since time immemorial by the indigenous people of the Northeast India. Capsicum species have been widely used in Northeastern states of India for the treatment of various ailments such as *Capsicum annum* fruits are used for gripping pain of the stomach and roots are used in asthma. *Capsicum frutescens* leaves paste are applied locally on joints, fruits are used in the treatment of tonsillitis, ulcer and dysentery.

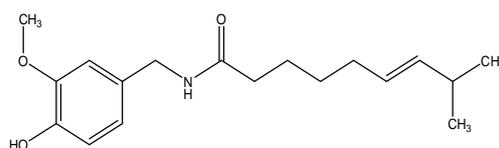
Table 1: Traditional uses of *Bhut jolokia*³

Parts of the plant used along with mode of administration	Disease used for
Low quantities of the ripe fruit consumed orally on regular basis	Asthma
Oral consumption of low quantity on regular basis of the fruit	Gastro-intestinal abnormalities
Hot infusions of fruits are applied locally (not to be applied on injured tissue).	Toothache and muscle pain
Paste of tender leaves is applied locally	Removal of puss from boils
Paste of leaves is applied locally	Arthritis

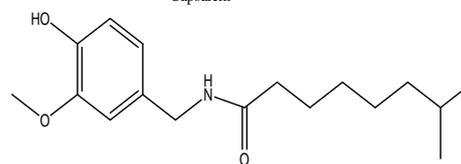
Chilli peppers are used as spice in many parts of the World because they attribute colour, pungency and flavour to food.⁵ *Bhut jolokia* is used as food and spice, as well as an unexpected remedy to summer. Besides having medicinal uses the fruit is pickled and used for flavouring curries.

Chemical composition

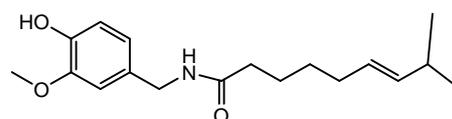
Capsicum fruits represent a group of five characteristic pungent compounds, which are collectively termed as capsaicinoids and are produced as secondary metabolites by chilli peppers, probably as deterrents against certain mammals and fungi. These are named as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homohydrocapsaicin. Chemically, these are acid amides of vanillylamine with C9-C12 branched chain fatty acid. Capsaicinoids are synthesized and accumulated in the epidermal tissue of the placenta.⁶ Capsaicin and dihydrocapsaicin together constitute more than 80% of total capsaicinoids content of capsicum.⁷ The capsaicinoids (mainly capsaicin) content generally indicates the hotness or pungency of capsicum (*chilli*). This also ensures the functional as well as nutritional quality of capsicum. Biosynthesis of capsaicin depends on the gene *AT3*, which resides at the *pun1* locus, and which encodes a putative acyltransferase.⁸



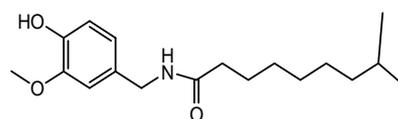
Capsaicin



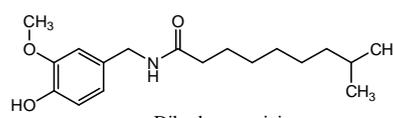
Nordihydrocapsaicin



Homocapsaicin



Homohydrocapsaicin



Dihydrocapsaicin

Bhut jolokia contains more capsaicin (3-5%) as compared to any other Indian chilli. Tiwari et al (2005) studied capsicum plants with considerable content of capsaicin which drops to 50% if grown in central India rather than

Northeastern India. This may be due the climatic factors of the region.⁹ It has been suggested that the geographical and climatic factors could affect the capsaicinoids content of capsicum grown in a particular geographical location.¹⁰ The capsaicin found in capsicum species has been reported to have various pharmacological activities and some of the clinical applications are as follows:

Pain reliever

It has been reported that capsaicin helps in reducing inflammatory heat and noxious chemical hyperalgesia and pain from rheumatoid arthritis or fibromyalgia.^{11, 12}

Cancer prevention

Studies reveal that Capsaicin represses the growth of various malignant cell lines by induction of cycle arrest, apoptosis, autophagy and also by the inhibition of cellular metabolic activation.^{11,13-16} It has been found that capsaicin was able to block breast cancer cell migration and kill prostate cancer cells, and dihydrocapsaicin was reported to induce the autophagy in HCT116 human colon cancer cells.^{11,17-19} The metabolites of capsaicin (such as the reactive phenoxy radicals) can even attack the DNA and trigger the mutagenicity and malignant transformation^{11,20}.

Cardiovascular activity

Capsaicin can reduce the incidence of cardiovascular diseases by inhibition of platelet aggregation and the activity of clotting factors VIII and IX. Capsaicin can pass through plasma membrane of platelets and thus alter membrane fluidity.^{11,21-22} It has been reported that capsaicinoids have potential beneficial effects on the cardiovascular system to treat various cardiovascular threats in human beings that include coronary heart disease, myocardial infarction, hypertension and atherosclerosis.^{11,23-24} Studies reveal that capsaicin has been able to increase the resistance of LDL to oxidation by delaying the initiation of oxidation and slowing the rate of oxidation. Consumption of chilli regularly for 4 weeks can increase the resistance of serum lipoproteins to oxidation in adult men and women.^{11,25}

Reduction of weight

Capsaicin was found to reduce body fat accumulation in animal experiments as well as clinical studies. The antiobesity effect of capsaicin showed that thermogenesis and lipid metabolism related proteins were markedly altered upon capsaicin treatment. The non-pungent CH-19 sweet pepper is responsible for weight loss. Studies reveal that body temperature and oxygen consumption increase with a single dose of CH-19 sweet pepper and regular intake of it can promote reduction of body weight and oxidation of body fat.^{11,25}

Gastrointestinal benefits

Capsaicin-sensitive sensory nerves are also present in gastrointestinal system which plays a crucial role in

maintenance of gastrointestinal mucosa integrity against injurious interventions. Capsaicinoids exert either beneficial or detrimental effects on gastrointestinal mucosa depending on the dose and/or duration of drug treatment.¹¹

Inhibition of bacterial growth

It was reported that capsaicin showed an in vitro anti-*H.pyroli* against MtzR and MtzS strains, thus treatment with capsaicin may be a useful treatment for antibiotic resistant strains and for patients who do not wish to take synthetic antibiotics.²⁷

Antiinflammatory property

It was found that the effect of capsaicin on the production of inflammatory molecules in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages showed significant inhibition of production of LPS-induced PGE2 in a dose-dependent manner. Capsaicin did not affect the COX-2 expression at either the protein or mRNA level, but inhibited the enzyme activity of COX-2 and the expression of the iNOS protein. It was reported that the anti-inflammatory action of capsaicin may occur through a novel mechanism, not by a VR-1 receptor-mediated one.²⁸

Antiplatelet effect

Capsaicin is a potent inhibitor of platelet aggregation and release reaction. Capsaicin has been reported to inhibit platelet aggregation and the activity of clotting factors VIII and IX, a property which causes reduction of the incidence of cardiovascular diseases. Capsaicin can reduce the hemolysis of RBCs induced by hydrogen peroxide or hypo tonicity. Capsaicin have some membrane stabilizing property and this might lead to the interference of the activation of phospholipase A2.²⁹

Antioxidant activity

Capsaicin has antioxidant property. The antioxidant activity of capsaicin was reported in the oxidation of methyl linoleate (ML) in homogeneous solution, ML micelles in aqueous dispersions and also of soybean phosphatidylcholine liposomal membrane, and was compared to that of alpha-tocopherol (alpha-TOH) which is one of the most important antioxidants in vivo.³⁰ The antioxidant property of capsaicin can even prevent cardiovascular diseases.

Hepatoprotective effects

Capsaicin has shown hepatoprotective activity against carbon tetrachloride (CCl₄)-induced liver injury in rats.³¹

Antidiabetic activity

Capsaicin is also reported to have antidiabetic activity. Antidiabetic effect of caffeine and capsaicin on the blood glucose level of obese/diabetic model mice was found to decrease the blood glucose level of KK-A(y) obese/diabetic mice.³²



The pungent principle of *Bhut jolokia*

The hotness or pungency is expressed commonly in terms of Scoville Heat Units (SHU) with respect to the total capsaicinoids content of capsicum. The hot sensation of *Bhut jolokia* is caused by a compound 'capsaicin' localized mainly in the placenta and the flesh of a chilli fruit. It survives both cooking and freezing temperature and dissolves in fat. It is detectable to the human taste buds to one part in one million, this powerful alkaloid is extracted from chillies as an oleoresin (thick oil). The oleoresin is refined into a white crystalline powder which is the pure capsaicin. The measurement of heat of a chilli was originally done by tasting a dilute solution of chilli extract and giving a value to it. In 1902, Wilbur Scoville had developed this method and so the heat value was expressed as Scoville Units. In Scoville's method, a measured amount of alcohol extract of the capsaicin oil of the dried pepper is produced, after which a solution of sugar and water is added incrementally until the "heat" is just barely detectable by a panel of (usually five) tasters; the degree of dilution gives its measure on the Scoville scale. A sweet pepper or a bell pepper, containing no capsaicin at all which has a Scoville rating of zero, meaning no heat detectable.³³ The heat of chilli is tested using spectrometer or HPLC (High Pressure Liquid Chromatograph) to rate the chillies in Scoville units which indicate parts per million of capsaicin. The Scoville units of pure capsaicin are between 150,00,000-160,00,000.³³

Recent Researches on *Bhut jolokia*

Interspecific hybrid

Bhut jolokia is known as the hottest chilli in the World displacing Red Savina until it was superseded by the Trinidad Moruga Scorpion in 2012. In 2007, Guinness World Records certified the Ghost Pepper (*Bhut jolokia*) as the World's hottest chilli pepper, 400 times hotter than Tabasco sauce.

Bosland and Baral et al reported that it is a naturally occurring inter specific hybrid. Interspecific hybridization between plant species in nature is a common phenomena that allows a species to introgress novel genes from another species. With introgressive hybridization, genes of one species move into another through the process of interspecific hybridization followed by successive backcrosses to one of the parents.

The taxonomic relationship of *Bhut jolokia* is based on RAPD markers which placed *Bhut jolokia* in a taxonomic position between *C. chinense* and *C. frutescens*, *Bhut jolokia* clustering more closely with *C. chinense* group. The average genetic similarity was found to be 0.79 between *C. chinense* and *Bhut jolokia* which is close to the 0.82 genetic similarity shared by the *C. chinense* accessions tested.³⁴

Antioxidant and anti-inflammatory activity

Bhut jolokia contains Capsaicinoids which possess antioxidant and antiinflammatory activities. Capsaicin

and dihydrocapsaicin (DHC) found in *Bhut jolokia* was compared with commonly consumed hot peppers, Jalapeno (*Capsicum annuum*) and Scotch Bonnet (*Capsicum chinense*). The concentration of Capsaicin and DHC in *Bhut jolokia* was 5.36%, which is about 338 and 18 times greater than in Scotch Bonnet and Jalapeno, respectively. For the first time report of the quantification of capsaicin and DHC in *Bhut jolokia* was done, also comparison of capsaicinoids content in *Bhut jolokia* with Jalapeno and Scotch Bonnet hot peppers and the COX and LPO inhibitory activities of capsaicin and DHC has been done.³⁵

Capsaicinoids Content

A comparative study among various chillies found in Assam has been reported such as *Capsicum annum* (Jati Jolokia), *Capsicum baccatum* (Ohm Jolokia), *Capsicum chinense* (*Bhut jolokia*), *Capsicum frutescens* (Dhan Jolokia, Maam Jolokia, Totta Bias) and *Capsicum pubescens* (Bhikue Jolokia). Amongst of all the capsaicinoids content of various chillies, *Bhut jolokia* capsaicinoid content (2.45%) was the highest. Different varieties of capsicum with decreasing order of their capsaicinoids content are as follows: *Bhut jolokia* (2.45%) > Dhan Jolokia (2.14%) > Maam Jolokia (1.38%) > Bhikue Jolokia (0.92) > Ohm Jolokia (0.67%) > Jati Jolokia (0.51%) > Totta bias (0.25%).³⁶

Pricalnical study on the gastric ulcer protective activity

Evaluation on the ulcer protective activity of the ethanolic extract of *Capsicum chinense* Jacq and Ranitidine on aspirin induced gastric ulcer on albino mice were performed. The study showed *Capsicum chinense* Jacq has an ulcer protective effect similar to that of Ranitidine.³⁷

CONCLUSION

Bhut jolokia is known Worldwide for its heat value and aroma which is indigenous to the Northeastern states of India. Commercialization of the plant had already been in the past few years which is good source of income for local farmers but the medicinal value of the plant has not been scientifically explored thoroughly. Once explored thoroughly in respects of medicinal value, it will cut the niche in the international market.

REFERENCES

1. Meghvansi MK, Siddiqui S, Khan MH, Gupta VK, Vairale MG, Gogoi HK, Singh L, Naga chilli: A potential source of capsaicinoids with broad-spectrum ethno pharmacological applications, Journal of Ethnopharmacology, 132, 2010, 1-14.
2. Perry L, Starch fossils and the domestication and dispersal of chili pepper (*Capsicum* spp. L.) in the Americas, Science, 315, 2007, 986-988.
3. Bhagowati RR, Chankija S, Genetic variability and traditional practices in Naga King Chilli Landraces of Nagaland, Asian Agri-History, 3, 2009, 171-180.



4. Sanatombi K, Sharma JG, Capsaicin Content and Pungency of Different Capsicum spp. Cultivars, Hortscience, 36, 2008, 89–90.
5. Pino J, Gonzalez M, Ceballos L, Centurio'n-Yah AR, M Trujillo-Aguirre J, Latournerie-Moreno L, Sauri-Duch E, Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (*Capsicum chinense* Jack.) cultivars grown in Yucatan, Food Chemistry, 104 , 2007, 1682–1686.
6. Iwai K, Suzuki T, Fujiwake H, Formation and accumulation of pungent principle of hot pepper fruits, capsaicin and its analogues in annum var. annum cv. Karayatsubusa at different growth stages flowering, Agricultural and Biological Chemistry, 43, 1979, 2493–2498.
7. Kirschbaum T, Mueller SP, Petz ME, Pungency in paprika (*Capsicum annum*), Heterogeneity of capsaicinoid content in individual fruits from one plant, Journal of Agricultural and Food Chemistry, 50, 2002, 1264–1266.
8. Stewart C, Kang BC, Liu K, The Pun1 gene for pungency in pepper encodes a putative acyltransferase, Plant Journal, 42, 5, 2005 675–88.
9. Tiwari A, Kaushik MP, Pandey KS, Dangi RS, Adaptibility and production of hottest chilli variety under Gwalior climatic condition, Current Science, 88, 2005, 1545-1546.
10. Antonius G F, Terry B and Jarret RL, Pungency in *Capsicum chinense*: Variation among countries of origin, Journal of Environmental Science and Health, 44, 2009, 174-184.
11. Arora R, Gill NS, Chauhan G, Rana AC, An Overview about Versatile Molecule Capsaicin, International Journal of Pharmaceutical Sciences and Drug Research, 3, 2011, 280-286.
12. Fraenkel L, Jr Bogardus ST, Concato J, Wittink DR, Treatment options in knee osteoarthritis: the patient's perspective, Archives of Internal Medicine, 164, 2004, 1299–1304.
13. Choi CH, Jung YK, Oh S, Selective induction of catalase mediated autophagy by dihydrocapsaicin in lung cell lines, Free Radical Biology and Medicine, 49, 2009, 245–257.
14. Ghosh AK, Basu S, Fas-associated factor 1 is a negative regulator in capsaicin induced cancer cell apoptosis, Cancer Letters, 287, 2010, 142–149.
15. Oh S, Choi CH, Jung YK, Autophagy induction by capsaicin in malignant human breast cells is modulated by p38 and ERK mitogen-activated protein kinase and retards cell death by suppressing endoplasmic reticulum stress-mediated apoptosis, Molecular Pharmacology, 78, 2010, 114–125.
16. Zhang J, Nagasaki M, Tanaka Y, Morikawa S, Capsaicin inhibits growth of adult T-cell leukemia cells, Leukemia Research, 27, 2003, 275–283.
17. Oh S, Choi CH, Jung YK, Autophagy induction by capsaicin in malignant human breast cells is modulated by p38 and ERK mitogen-activated protein kinase and retards cell death by suppressing endoplasmic reticulum stress-mediated apoptosis, Molecular Pharmacology, 78, 2010, 114–125.
18. Thoennissen NH, O'Kelly J, Lu D, Iwanski GB, La DT, Abbassi S, Leiter A, Karlan B, Mehta R, Koeffler HP, Capsaicin causes cellcycle arrest and apoptosis in ER-positive and negative breast cancer cells by modulating the EGFR/HER-2 pathway, Oncogene, 29, 2010, 285–296.
19. Yang ZH, Wang XH, Wang HP, Hu LQ, Zheng XM, Li SW, Capsaicin mediates cell death in bladder cancer T24 cells through reactive oxygen species production and mitochondrial depolarization, Urology, 75, 2010, 735–741.
20. Baez S, Tsuchiya Y, Calvo A, Pruyas M, Nakamura K, Kiyohara C, Oyama M, Yamamoto M, Yamamoto M, Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women, World Journal of Gastroenterology, 16, 2010, 372–378.
21. Zhou Z, Peng J, Wang CJ, Li D, Li TT, Hu CP, Chen XP, Li YJ, Accelerated senescence of endothelial progenitor cells in hypertension is related to the reduction of calcitonin gene-related peptide, Journal of Hypertension, 28, 2010, 931–939.
22. Adams MJ, Ahuja KD, Geraghty DP, Effect of capsaicin and dihydrocapsaicin on in vitro blood coagulation and platelet aggregation, Thrombosis Research, 124, 2009, 721–723.
23. Harada N, Okajima K, Effects of capsaicin and isoflavone on blood pressure and serum levels of insulin-like growth factor-I in normotensive and hypertensive volunteers with alopecia, Bioscience Biotechnology and Biochemistry, 73, 2009, 1456–1459.
24. Peng J, Li YJ, The vanilloid receptor TRPV1: role in cardiovascular and gastrointestinal protection, European Journal of Pharmacology, 627, 2010, 1–7.
25. Ahuja KD, Ball MJ; Effects of daily ingestion of chilli on serum lipoprotein oxidation in adult men and women, British Journal of Nutrition, 96, 2006, 239–242.
26. Leung FW, Capsaicin-sensitive intestinal mucosal afferent mechanism and body fat distribution, Life Science, 83, 2008, 1–5.
27. Zeyrek Y F, Oguz E, Invitro activity of capsaicin against *Helicobacter pylori*, Annals of Microbiology, 55, 125-127, 2005.
28. Kim CS, Kawada T, Kim BS, Han IS, Choe SY, Kurata T, Yu R, Capsaicin exhibits anti-inflammatory property by inhibiting IκB-α degradation in LPS-stimulated peritoneal macrophages, Cell Signal, 15, 2003, 299-306.
29. Wang JP, Hsu MF, Teng CM, Antiplatelet effect of capsaicin, Thrombosis Research, 36, 1984, 497-507.
30. Okada Y, Okajima H, Antioxidant effect of capsaicin on lipid peroxidation in homogeneous solution, micelle dispersions and liposomal membranes, Redox Report, 6, 2001, 117-22.
31. Hassan MH, Edfawy M, Mansour A, Hamed AA, Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats, Toxicology and Health, 28, 2012, 428-438.
32. Okumura T, Tsukui T, Hosokawa M, Miyashita K, Effect of caffeine and capsaicin on the blood glucose levels of obese/ diabetic KK-A(y) mice, Journal of Oleo Science, 61, 2012, 515-523.



33. Scoville WL, Note on capsicum, Journal of the American Pharmaceutical Association, 1, 1912, 453-454.
34. Bosland WP and Baral BJ, *Bhut jolokia*: The World's Hottest Known Chile Pepper is a Putative Naturally Occurring Interspecific Hybrid, Hortscience, 42, 2007, 222–222.
35. Liu Y, Nair MG, Capsaicinoids in the hottest pepper *Bhut jolokia* and its antioxidant and antiinflammatory activities, Natural product communications, 5, 2010, 91-94
36. Sarwa K, Mazumder B, Rudrapal M, Debnath M, Kumar A, Verma KM, Jangdey SM, Capsaicinoids Content of Some Indigenous Capsicum Varieties of Assam, India, Journal of Natural Sciences Research, 3, 4, 2013, 112-116.
37. Das S, Deka S, Gohain K, A preclinical study on gastric ulcer protective activity of world's hottest chilli *Capsicum frutescenes*, Journal of Clinical and Diagnostic Research, 2, 2008, 1024-1027.

Source of Support: Nil, Conflict of Interest: None.

