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# Extraction and Comparative Analysis of Moisture and Capsaicin Contents of *Capsicum* Peppers

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**Abstract**

Capsaicin and moisture contents of three varieties of *Capsicum* peppers [*Capsicum frutescens*- Bird eye pepper (X1) and two varieties of *Capsicum annum*: Chilli pepper (X2) and sweet pepper (X3)] were determined and the level of pungency related to reported observed effects on human-beings especially, as a topical analgesic. Extraction was carried out using modified method described by Kosuge et al (1958). Fractions obtained were identified as capsaicin by direct comparison with authentic samples and their ir with literature data. Every 50 g of *Capsicum* pepper gave mean capsaicin extracts as 0.206 ± 0.02 g (X1) and 0.066 ± 0.01 g (X2); X3, was in trace amount (<0.001 ± 0.00 g). Values suggest species-specific relationship in capsaicin content and composition. The Pearson's Product Moment Correlation Coefficient (r) between the moisture and capsaicin contents showed moderate negative correlation. The relationship was not statistically significant (p>0.05). Moisture contents was higher in two varieties- X2 (66.97 ± 0.05%) and X1 (51.57 ± 0.03%); X3 had the lowest moisture content of 43.19 ± 0.01% suggesting that moisture content and not size affects the level of pungency contrary to popular believe that the bigger pepper are, the hotter. Bird-eye and chilli peppers, with high capsaicin content, would have medicinal values as topical analgesic.

## Keyword

Capsaicin; *Capsicum* pepper; *C.frutescens*; *C.annum;* Topical analgesic

## Introduction

Medicinal plants are major sources of drugs on the basis of their phytochemical composition. Medicinal plants typically contain bioactive compounds that may be pharmacologically active individually, additively or in synergy to improve health [1,2]. They exhibit varied biological activities like anti-inflammatory antifungal, antibacterial, analgesic, antioxidant, anticancer and several other pharmacological actions. The molecules are usually synthesized for clinical uses. *Capsicum* peppers are not exceptions to some of these properties.

The *Capsicum annum* and *Capsicum frutescens* are two commonest species of *Capsicum* peppers. The *Capsicum* peppers though commonly used as food additives have several medicinal values [3-5] that should be exploited. Pungency is produced by the capsaicinoids, alkaloid compounds, often called capsaicin after the most pungent and prevalent one [6]. Capsaicin, the aromatic pungent non-volatile phenolic compound [3] with molecular structure: ($C_{18}H_{23}NO_3$ )-8-methyl-N-Vanilyl-1, 6-nonenamide majorly lends credence to the numerous medicinal values of red pepper in alternative medicine as a 'pain management medicine'.

Topical analgesics have comparatively small market share compared with over- the-counter analgesics suggesting a limited use of topical agents with different topical options for pain management even though nature has abundant source of topical analgesics which according to Peppin et al. [7] have more desirable side effect profile than orally, transdermally, parenterally, or intrathecally administered analgesics. The substance capsaicin gives *Capsicum* peppers their intensity when ingested or topically applied.

Various methods for the isolation of the pungent agent, capsaicin, has been recorded using single species of both dried red (ripe) fruits and fresh green peppers in each experiment as the source of the active principle [8,9]. Apart from the fact that some of these methods used only single species and single variety, they used chlorinated hydrocarbon as solvent in the extraction of capsaicin which are implicated by the National Cancer Institute as causes of teratogenesis [10]. Additionally, all these methods require expensive equipment and reagents which may not be available in many laboratories or institutions in developing countries like Nigeria and Africa as a whole. Cultivated plant cells and tissues derived from hot pepper fruits have been used in an attempt to produce high quantities of capsaicin for the pharmaceutical and food industries [11].

In an attempt to extract and compare the pungent principle, the work endeavored to utilize three varieties of two common species of freshly ground red (ripe) capsaicin peppers as the source of the active principle using a modified existing extraction method by Kosugue et al. [9], compare pungency based on the extracted capsaicin contents of the different species of pepper and relate the level of pungency to reported observed effects on human beings and proffer recommendations of the species best used as medicinal/drug *Capsicum* and relate moisture content to the level of pungency. It is believed that the study will increase awareness of this potent plant with its numerous and divergent usefulness. It will help plant breeders to selectively develop cultivars with high degree of pungency for use in the pharmaceutical especially for relieve of pain by itself or in synergy with other topical agents.

## Materials and Methods

Three varieties of pepper -sweet pepper (*Capsicum annum*), chili (*Capsicum annum)* and bird-eye pepper (*Capsicum frutescens)* were bought from the local market. All samples used were bought fresh and ripe. They were washed and air dried for 30 minutes, then chopped into pieces and oven dried at a temperature of 60°Cfor 12 h. The dried samples were ground separately, to fine powder with the aid of national electric blender and stored in labeled polyethylene bags.

Authentic and standard samples of capsaicin compounds were obtained from reliable commercial source and used for comparison.

### Moisture content

Fresh samples were Washed air dried for 30min and weighed. The weight was recorded as the initial (wet) weight. They were chopped into pieces and over dried at a temperature of 60°C for 12 h to attain a constant weight (the dry weight). The difference between the wet and dried samples was considered as the moisture content. Statistical analysis chi-square and Pearson's Karl co-efficient correlation were used to determine the association between capsaicin and other variables.

### Extraction of capsaicin

Extraction of capsaicin was carried out using a modified method as described by Kosugue et al [9]. 50 g of ground dried red pepper were extracted by agitation for 2 h in 150 ml acetone and allowed to stand until the particulate matter settled. The acetone layer was decanted (Extract 1). The process was repeated until the acetone was almost colourless. The insoluble residue (Residue 1) was filtered to dryness using a Buchnar funnel under vacuum. Residue 1 was a cream coloured fine powder. Extract 1 and the filtrate from and the filtrate from residue 1 were pooled together and evaporated to dryness (Residue 2). To this, 30 ml of acetone was added and the mixture evaporated to dryness (Extract 2). This was the oleoresin *Capsicum.*

To this, 5% sodium trioxocarbonate (iv) and ether, 30 ml of each, twice were added. Phase separation was carried out in a separator funnel and the ether extracts combined (Extract 3). The water layer was discarded while Extract 3 (ether layer) was evaporated to dryness (Residue 3). To this, 30 ml of acetone was added and the mixture filtered to give an acetone solution (Extract 4) and a residue (Residue 4). Extract 4 was evaporated to dryness. Residue 5 was obtained; it was then treated again with 30 ml of ether and then 30 ml of 5% NaOH twice. After 6 h, phase separation was conducted in a separatory funnel to give a water layer (Extract 5) and an ether layer (Extract 6).

To Extract 5, 5% HCl was added to readjust the pH to 7.0 and the fatty float on the water extracted with 30 ml ether, two times. This gave another ether layer (Extract7) and a water layer (Extract 8). Extract 6 and 7 were combined together and evaporated to dryness (Residue 6) was treated with 10 ml of acetone and filtered to give Extract 9 and Residue 7. Extract 9 was evaporated to dryness to give a phenolic substance (Residue 8). Extraction of Residue 8 was carried out using petroleum ether. The resultant petroleum ether extract is the crude capsaicin.

### Statistical analysis

Data were expressed as the mean ± SEM and percentages. The nature of the relationship between capsaicin content of the different *Capsicum* peppers and their moisture contents were done using the Pearson product moment correlation coefficient (r). All data were considered significant at $p<0.05$.

## Results

Moisture and capsaicin content in the three different species of *Capsicum* pepper evaluated showed variations.

Analysis of moisture content of the three varieties of *Capsicum* pepper showed that sweet pepper had the least moisture content of 43.19% ± 0.01 followed by bird-eye, 51.57% ± 0.03 and chilli, 66.97% ± 0.05 (Table 1). The mean capsaicin content of sweet pepper was found to be the least, in trace amount (<0.001 ± 0.00 g) while the capsaicin content of bird-eye pepper was higher (0.206 g ± 0.02) than that of chilli pepper (0.066 ± 0.01 g).

The weights of the capsaicin extracted from the three samples increased significantly ($p<0.05$) from sample X3, X2 to X1 while size increased significantly ($p<0.05$) from X1, X2 to X3 indicating an inverse relationship between size and pungency. There was a moderate negative correlation ($r = -0.639$, $p<0.05$) between the moisture content and the capsaicin content implying that moisture content does not play a vital role in the capsaicin content of *Capsicum* peppers.

| Sample | Moisture content of sample (%) | Capsaicin Content/ 50 g | Length (Cm) | Width (Cm) |
|---|---|---|---|---|
| Bird-eye pepper (X1) | 51.57 ± 0.03 | 0.206 ± 0.02 | 2.5 ± 0.43 | 0.5 ± 0.07 |
| Chilli pepper (X2) | 66.97 ± 0.05 | 0.066 ± 0.01 | 3.1 ± 0.14 | 2.96 ± 0.35 |
| Sweet pepper (X3) | 43.19 ± 0.01 | <0.001 ± 0.00 | 9.1 ± 0.22 | 3.8 ± 0.16 |
| Values are mean of triplicate determinations. | | | | |

**Table 1:** Moisture and capsaicin extracts of bird-eye, chilli and sweet peppers.

The infrared spectroscope compound analyses (Table 2) showed the presence of phenol, amide, olefins and aromatic functional groups having the following stretches and bands: IR Vmax {3550-3100 (broad/w O-H), 1780-1700 (C=O), 1620 (C=C) and 1520-1580 (C=C)}$cm^{-1}$ respectively. The capsaicin obtained from sweet pepper was in trace amount.

| Absorption Band ($cm^{-1}$) | Intensity | Group | Remark |
|---|---|---|---|
| 3100-3550 | Br/w | OH | Phenolic O-H stretching vibrations. |
| 1700-1780 | W | C=O | C=O stretching vibrations of amides. |
| 1620 | W | C=C | C=C stretching vibrations in olefins. |
| 1520-1580 | W | C=C | C=C stretching (aromatic) |
| The correlation (r) value was -1.0 (p>0.05) | | | |

**Table 2:** Ir Analysis of Samples X1, X2 and X standard.

## Discussion

The capsaicin content of *Capsicum* peppers is pirmarily, responsible for the many values of Capsicum peppers [3].

Capsaicin extracted from the various species of *Capsicum* peppers were identical. The IR spectra of the capsaicin crude extracts were identical with that of standard and the characteristic frequencies reported by Muller-Stock et al. The close similarities in the respective IR spectrum suggest that the isolates have basic similar structures and all isolates are in close resemblance with the standard. The weak intensity could be as a result of the dilute nature of the crude extract.

The inverse relationship between size and pungency confirms report by Derek and Wibberley that peppers are hotter if they are smaller. The results contradict popular belief that the bigger the 'hotter'. Sweet pepper, though the biggest had only trace amount of capsaicin. Capsaicin is known to be the basic chemical that is tested for when determining the piquancy of peppers. It is very potent to the taste and nerves. The amount of capsaicin will determine the level of pungency.

Pungency depends on and is controlled by the gene AT3 which resides at the Pun 1 locus that encodes a putative acyltransferase but sweet pepper rids itself of capsaicin with its recessive genes which results in non-pungency. Lots of the capsaicin in the pungent (hot) pepper is primarily found within the fruits of *Capsicum* species [6] accumulated in the placenta [12] and their concentration increases during ripening [13]; pepper becomes hotter as they ripen [3]. This is more noticeable in some species like the bird-eye and chilli peppers with high capsaicin content and hence, high Sconville units [14,15]. The study indicates that the *C. frutesence* is more pungent than members of the *C. annum* in line with Simon et al. [6]. The high pungency of the *C. frutesence* would classify them as hot spices and thus, drug/medicinal *Capsicum* [16]. Chilli pepper (X2), though a member of the *C. annum* is midly pungent; with its pungency, it could be used as substitute for the similar functions performed by bird-eye pepper (X1) in the class, *C. frutesence.* This shows an overlap of functions within these two classes as against Heiser's strict classification that restricts drug/medicinal Capsicums to members of *C. frutesence*. The usefulness of the samples X1 and X2 as medicinal drugs has been reported by Arnold et al. [4,17]. Samples X1 and X2 would be best used externally as counter-irritant for the treatment of arthritis, relief of pains and aches rheumatism, lumbago and neuralgia Homes et al. as an analgesic [18] and/or anti-inflammatory. It acts by selectively binding to a protein called, TRPVI [19], a heat activated calcium channel that resides on the membranes of pain and heat sensing neurons [20]. The capsaicin sensation overwhelms the nerves through calcium influx thereby blocking pain sensation from the nerves for some time. The mechanism of action involves capsaicin acting on efferent sensory neurons involved in pain receptors which are used for analgesics. This makes them useful as counter-irritant and for the treatment of headaches. By binding to the TRPVI receptors on Aδ and C fibers, it causes the release of substance P and calcitonin gene-related peptide [21,22] the capsaicin molecule then produces similar sensations to those of excessive heat. This explains the reasons for the spiciness of capsaicin being described as a burning sensation. The results of both human and animal nerve biopsy studies demonstrate nerve fiber degeneration beneath the capsaicin application site. Such degeneration may be one of the mechanisms of pain relief.

Capsaicin, when applied topically, does not result in significant systemic accumulation except for application-site reactions like pain and eryththema; the incidence of burning may decrease with repeated use; however, the frequent occurrence of this side effect may negatively affect treatment adherence and consequently the patient's ability to benefit from its use [21]. When capsaicin is compounded in a topical preparation at high concentrations and administered as a single application, the analgesic benefit appears to last for several weeks. Currently, topical liquid preparations of 10% and 20% capsaicin are in phased clinical trials [23]. There has been interest in using capsaicin in a number of neuropathic pain disorders such as DPPN, human immunodeficiency virus neuropathy (HIVN), and PHN.

Chronic exposure to capsaicin causes reduction of neuro-transmissions by the neuron leading to decrease pain sensation and blockade of neurogenic inflammation. This lends its usefulness as topical analgesic in the treatment of post-surgical and oesto-athritic pain [24]. Thus, capsaicin acts like naloxone and other compounds that compete for receptor sites with endorphins and opiates and cause blockade [25]. The alkaloid content could be used as a source of synthetic heliotrope, as a therapeutic agent particularly as a chemoreceptor against mutagenesis or tumor genesis [26]. Capsaicin might actually protect against peptic ulcer by stimulating the hormone that increase blood floor and nourishes gastric mucosal membrane [27].

Low levels of moisture contents for bird-eye and sweet pepper with the former having the least moisture content could be attributed to its fibrous nature which is 'slightly woody'. Bird-eye pepper shares the same fibrous characteristic though to a lesser extent. On the other hand the chilli peppers tend to be fleshy, such characteristics seems to make them richer in moisture content hence their high moisture content. It is also known that the lower the moisture content of a given plant product, the lower the rate of deterioration the implication of this would be that, bird-eye pepper would have a longer shelf life than chilli pepper; this characteristic in addition to its high capsaicin content would make it the best specie of choice as a topical agent. The moderate negative correlation between capsaicin and moisture contents of the *Capsicum* peppers suggests that moisture content does not play a vital role for the 'hotness' (capsaicin content) of peppers.

The capsaicin contents were in line with the works of as reported by Suzuki and Iwai [12], that capsaicin content was higher in *C. frutescence* than in *C. annum*, respectively. The method of extraction was thus successful because without performing a sufficient extraction of capsaicin from the starting materials satisfactory results cannot be obtained.

Species-specific relationships in capsaicin contents and composition exist. Results showing differences in capsaicin contents between pungent and sweet types of pepper have similarly been reported by Quagliotti and Ottaviano.

Thus, the determination of capsaicin is not only useful for taxonomical identification of individual species but also in helping determine the species that can be crossbred to give hotter specie and their classification as medicinal/drug *Capsicum.*

## Conclusion

Pungency (capsaicin content) of *Capsicum* peppers is dependent on variety and not the moisture or 'fleshy' nature of *Capsicum* peppers. The determination of capsaicin content is useful for taxonomical identification of individual species suggesting specie-specific relationship and forms basis for cross breeding characteristics/traits. It also lends credence for the classification of *Capsicum* peppers according to their uses/usefulness as medicine for topical treatment by itself or in synergy with other topical agents in a topical polyphamacology.

The IR analysis though it serves the purpose of giving an idea of the structure of the compound is not enough to elucidate structure of compounds so other instrumentation techniques could be employed for the structure of the compound to be fully elucidated. This method could be useful in developing countries where sophisticated equipment are rare to come by, costly and most reagents lacking.

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