Chemist Amol Kadam Art 2018-19

Asian J. Research Chem. 11(4): July – August 2018

ISSN 0974-4169 (Print)
0974-4150 (Online)

www.ajrconline.org

RESEARCH ARTICLE

Study of the effect of Sodium dodecyl sulphate (SDS) on isolation of Curcumin from oleoresin of Turmeric (*Curcuma longa*)

Amol A. Kadam*, Dr. Amit P. Pratap
Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai-86
*Corresponding Author E-mail: **kadamamol@rediffmail.com**

ABSTRACT:
This study was undertaken for extraction and isolation of Curcumin from *Curcuma longa* rhizomes. In the present study effect of sodium dodecyl sulphate on the isolation of Curcumin was carried out and it was found that it helps in the isolation of Curcumin from the oleoresin of *Curcuma longa*. The structure of the compound was elucidated and confirmed by thin layer chromatography, infrared spectroscopy, mass spectrometry and nuclear magnetic resonance spectral analysis. HPLC method was used for the identification and quantification of Curcumin by comparing with a standard.

KEYWORDS: Curcumin, Isolation, Sodium dodecyl sulphate (SDS).

INTRODUCTION:

Curcuma Longa (Turmeric) is a rhizomatous, herbaceous perennial plant ofZingiberaceae family, found mainly in the Indian subcontinent and Southeast Asia. The rhizome of the plant is known to be used extensively in the treatment of a runny nose, cough, antiseptic and so on[1,2]. It has also been reported to exhibit anticancer and antimicrobial activities [3,4]. The ethanolic extract of *Curcuma longa* has been reported to be antioxidant and found to have a potent scavenging activity reactive species as well as an inhibitory effect on LDL [5].

The phytochemical screening of *Curcuma longa* revealed the presence of Phenolic diketones (Curcuminoids), turmerones, -phellandrene, Zingiberene, sesquiterpenes and so on [6].The plant rhizome has been reported to possess mainly orange-yellowcolored pigment identified as Curcuminoids, mainly contain Curcumin as a major component.

Received on 27.04.2018 Modified on 15.05.2018
Accepted on 30.05.2018
Asian J. Research Chem. 2018; 11(4):713-716.
DOI: 10.5958/0974-4150.2018.00126.8

Curcumin is the major and the active ingredient of curcuminoids. In different turmeric varieties, the Curcumin content varies from 2-8%[7]. Pharmacological and clinical studies revealed that it has antioxidant[8–10], anti-inflammatory[11], antimicrobial[12], anti-mutagenic[13], and anticancer properties[14] etc. new experimental evidence have shown beneficial health effects of Curcumin. Despite the amazing therapeutic properties of Curcumin, it has a very poor solubility in an aqueous environment which reduces its usefulness as a therapeutic agent. Curcumin is also sensitive to the light and decomposes in alkaline solution but relatively stable in acidic medium.

Literature survey revealed that severalstudiesconducted on Curcumin extraction and isolation, but isolation of Curcumin is tedious work and need simple and efficient process. A number of isolation methods were listed in the literature for the Natural products[15–24]. Therefore, the purpose of the study was to develop a simple, easy and efficient method of extraction and isolation. The study involves the effect of sodium dodecyl sulphate on the isolation of Curcumin from the oleoresin. Also, the study involved the identification by TLC and quantification by using UV visible as well as HPLC method.

MATERIAL AND METHODS:

Plant Materials:

Turmeric powder was purchased from the local market (Moisture content 13%, as analyzed using LOD method and Dean-stark toluene distillation method reported in the literature)[25].

Chemicals and reagents:

Sodium dodecyl sulphate and acetone were purchased from the M/s Loba Chem Pvt. Ltd., Mumbai (India). HPLC grade acetonitrile, methanol and acetic acid were purchased from Merck India.

Extraction:

10 g of Turmeric powder was taken in a thimble and placed in a Soxhlet apparatus. 100 ml of Acetone was added to a flat bottom flask and set the heating power to 4-6 cycle per hour so that complete extraction achieved within 3 hrs. of extraction time (to ensure complete extraction). The yellow color acetone extract was obtained after complete extraction. The extract was cooled and transferred to 250 ml round bottom flask and concentrated in vacuo under reduced pressure to obtain orange-yellow viscous oil. Take the weight of the Curcumin oleoresin.

Isolation:

To the above viscous oily oleoresin, SDS solution (Prepared in water) was added. Addition of SDS solution results in the formation of a brownish solution. Above solution was Stirred for 2 hrs which results in the formation of a precipitate. This Precipitate was filtered and washed with 5 ml water. The reddish-brown color precipitate was obtained after drying.

Treatment is given to the above solid:

A) This reddish-brown precipitate was taken in 100 ml beaker and added 10 ml ethanol into it. The solution was boiled for 5 min and allow cooling at room temperature. Precipitate filtered and dried which again results in the light reddish brown precipitate.

B) The solid was taken in a 100 ml beaker and treated with the acidic solution having P^H below 2. It results in the formation of a yellow precipitate. The precipitate was filtered and dried.

UV Visible spectroscopy:

The Curcumin content of the Turmeric was found by Spectrophotometric analysis method[26,27]. The Spectrophotometric analysis was performed on UV-Visible Spectrophotometer Instrument (UV-1800, Shimadzu) at 425 nm. The standard calibration method applied within the range of 2 to 20 µg/ml. The Turmeric powder sample was extracted with Methanol and diluted to obtain absorbance in the range.

Identification of Curcumin by HPLC, NMR, IR and Mass spectroscopy:

HPLC analysis:

The HPLC system consisted of a Shimadzu UFLC prominence I HPLC system equipped with 20 µlinjector and UV detector and reverse phase C_{18} analytical column (150 X 4.6 id, 5 µm). The reagents for mobile phase preparation were of HPLC grade, and all mobile-phases used were filtered and degassed on a Millipore HPLC filtration system with 0.45 µm pore size membrane filters. Unless otherwise stated the HPLC mobile-phase consisted of Acetonitrile and 2% acetic acid(60:40) at a flow rate of 1.0 ml/min and detected at a wavelength of 425 nm [28]. The curcumin content of Turmeric was found by an external standard calibration method.

NMR, IR and Mass spectroscopy:

The isolated Curcumin was crystallized repeatedly by using ethyl acetate and pure crystals of Curcumin were collected. The melting point of crystallized Curcumin was determined and found to be 180℃. The crystallized Curcumin was analyzed by nuclear magnetic resonance spectroscopy (NMR- 300 MHz), Fourier transform infrared spectroscopy (FTIR, model alpha and Bruker) and mass spectrometry for confirming the chemical structure.

RESULTS AND DISCUSSION:

Extraction of Turmeric (*Curcuma Longa*) powder was carried out by using acetone due to its very good extraction efficiency, cost, easy recovery and more solubility of Curcumin in it. The surfactant can be used to extract as well as isolate natural product from the raw material. Use of surfactant increases the solubility of hydrophobic compounds into the water and due to which the isolation of compounds becomes very easy. Influenced by this literature survey, we formulated a number of experiments using surfactants solution (sodium dodecyl sulphate solution). As a result of which it was found that surfactant solution can be used for the isolation of Curcumin from the oleoresin. The yield of crude Curcumin by proposed method was found to be 2.4 % and on treatment with ethanol 0.8%. on treatment with the acidic solution below P^H 2 gives yield 1.8%

Different concentration of SDS solution was prepared in water and used for the isolation of Curcumin. When the SDS mixed with the Curcumin oleoresin it results in the formation of reddish-brown solid. Two different methods were used for the isolation using SDS. First, in which SDS solution was prepared and Curcumin oleoresin dissolved in ethanol was added to the SDS solution and second in which SDS solution was added into the Curcumin oleoresin and stirred to obtain Curcumin solid. In both the methods, reddish brown solid was obtained and the solid was treated with ethanol to improve the color of the Curcumin, but from the

literature, it was found that Curcumin forms electrostatic interaction take place between the Curcumin and SDS which is quite stable in the acidic P^H range (5.0>7.0>2.0).

Boruah and others (2013) explained the formation of the complex on the basis of the electrostatic interaction between the Curcumin and SDS[29,30]. The complex formation may involve electrostatic interaction between the head groups of the surfactants and the diketo moiety of Curcumin in the diketo tautomeric form of the dye and at higher surfactant concentrations, the diketo Curcumin converts rapidly to the keto-enol form to retain its conjugation. The strength of the complex was found to be higher with SDS and at P^H 5.0 to 7.0 but it decreases at P^H 2.0. hence due to which the obtained brownish solid was treated with the acidic solution below P^H 2 which results in the formation of yellow solid. It can be easily separated using simple filtration method.

The Curcumin was identified by TLC using Chloroform: Acetic acid (99:1) solvent system. The Curcuminwas confirmed by comparing with the standard. The Rf value of Curcumin, demethoxycurcumin and bisdemethoxycurcumin found to be 0.51, 0.32, 0.12 respectively from the TLC (Fig. 1).

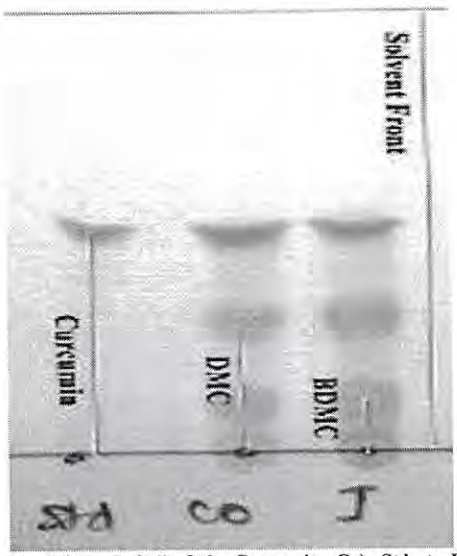

Figure 1: TLC of Std) Std. Curcumin Co) Std + Isolated CurcuminJ) isolated Curcumin

The Curcumin content of the Turmeric was found by Spectrophotometric analysis method[26,27]. The Curcumin content of the Turmeric powder was calculated and found to be 3.06%.

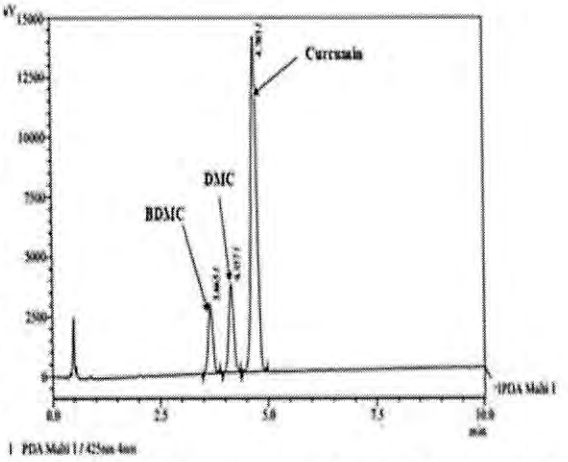

Figure 2: HPLC chromatogram of an extract of Turmeric Powder (*Curcuma longa*)

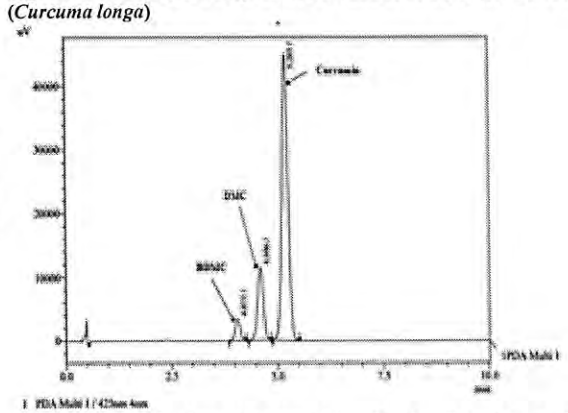

Figure 3: HPLC chromatogram of isolated solid after acidic treatment

The content of Curcumin was 2.89%, as determined by the HPLC peak area. The retention time of Curcumin, demethoxycurcumin, and bisdemethoxycurcumin are 5.9, 5.2, and 4.7 respectively. The Curcumin content of the extract and isolated material is 71.7% and 83.2% respectively.

The H^1 NMR spectrum of the isolated compound showed characteristic signals of Curcumin. The spectrum was matched with reference for structural confirmation [31]. ^1H NMR (300 MHz, $CDCl_3$) δ 16.04 (bs, 1H), 7.59 (d, J = 15.8 Hz, 2H), 7.13 (dd, J = 8.3, 1.9 Hz, 2H), 7.05 (d, 2H), 6.94 (d, J = 8.2 Hz, 2H), 6.48 (d, J = 15.8 Hz, 2H), 5.81 (s, 1H), 3.95 (s, 6H).

The mass spectra of the isolated compound showed a molecular ion peak at m/z 369.1 corresponding to molecular formula, $C_{21}H_{20}NO_6$ which is similar to the mass spectral data of Curcumin (Mol. Wt. 368) [32]. It also contains distinct peaks like 245.2, 191, 177.2, and 148.9 which are responsible for the fragmentation of Curcumin.

CONCLUSION:

The result of present study establishes the use of sodium dodecyl sulphate solution for the isolation of Curcumin from the oleoresin of the *Curcuma longa*. Formation of the complex can be break by the treatment of obtained solid with the acidic solution below P^H 2. This treatment does not result in the decomposition of Curcumin as all the analytical data matches with the standard Curcumin.

ACKNOWLEDGMENT:

The authors are grateful to the authorities of Institute of Chemical Technology, Mumbai for the facilities.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Parthasarathy VA, Chempakam B, Zachariah TJ. *Chemistry of Spices*. UK: CABI; 2008. doi:10.1079/9781845934057.0000.
2. Fallis A. *Turmeric Genus Curcuma*. Vol 53. Boca Raton, Florida, United States: CRC Press; 2013. doi:10.1017/CBO9781107415324.004.
3. Wu Y, Chen Y, Xu J, Lu L. Anticancer activities of curcumin on human Burkitt's lymphoma. *Zhonghua Zhong Liu Za Zhi*. 2002;24(4):348-352. https://www.ncbi.nlm.nih.gov/pubmed/12408761.
4. De R, Kundu P, Swarnakar S, et al. Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. *Antimicrob Agents Chemother*. 2009;53(4):1592-1597. doi:10.1128/AAC.01242-08.
5. Lee HS, Lee MJ, Kim H, et al. Curcumin inhibits TNFalpha-induced lectin-like oxidised LDL receptor-1 (LOX-1) expression and suppresses the inflammatory response in human umbilical vein endothelial cells (HUVECs) by an antioxidant mechanism. *J EnzymInhib Med Chem*. 2010;25(5):720-729. doi:10.3109/14756360903555274.
6. Yadav S, Khar R, Mujeeb M, Akhtar M, Yadav D. Turmeric (*Curcuma longa* L.): A promising spice for phytochemical and pharmacological activities. *Int J Green Pharm*. 2013;7(2):85-89. doi:10.4103/0973-8258.116375.
7. Sahne F, Mohammadi M, Najafpour GD, Moghadamnia AA. Enzyme-assisted ionic liquid extraction of bioactive compound from turmeric (*Curcuma longa* L.): Isolation, purification and analysis of curcumin. *Ind Crop Prod*. 2016;95:686-694. doi:10.1016/j.indcrop.2016.11.037.
8. El-Barbary MI. Detoxification and antioxidant effects of garlic and curcumin in Oreochromis niloticus injected with aflatoxin B(1) with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. *Fish PhysiolBiochem*. 2016;42(2):617-629. doi:10.1007/s10695-015-0164-4.
9. Ahmed HH, Shousha WG, Shalby AB, El-Mezayen HA, Ismaiel NN, Mahmoud NS. Curcumin: a unique antioxidant offers a multimechanistic approach for management of hepatocellular carcinoma in rat model. *Tumour Biol*. 2015;36(3):1667-1678. doi:10.1007/s13277-014-2767-2.
10. Gupta A, Rath PC. Curcumin, a natural antioxidant, acts as a noncompetitive inhibitor of human RNase L in presence of its cofactor 2-5A in vitro. *Biomed Res Int*. 2014;2014:817024. doi:10.1155/2014/817024.
11. Shakeri F, Boskabady MH. Anti-inflammatory, antioxidant, and immunomodulatory effects of curcumin in ovalbumin-sensitized rat. *Biofactors*. 2017;43(4):567-576. doi:10.1002/biof.1364.
12. De R, Kundu P, Swarnakar S, et al. Antimicrobial activity of curcumin against helicobacter pylori isolates from India and during infections in mice. *Antimicrob Agents Chemother*. 2009;53(4):1592-1597. doi:10.1128/AAC.01242-08.
13. Shukla Y, Arora A, Taneja P. Antimutagenic potential of curcumin on chromosomal aberrations in Wistar rats. *Mutat Res*. 2002;515(1-2):197-202.
14. Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway. *Cancer Res*. 2007;67(5):1988-1996. doi:10.1158/0008-5472.CAN-06-3066.
15. K. Kamalakannan, Umesh Kumar, Kumud Chandra Kandpal, Sunita Minz, Madhulika Pradhan AS and TS. Isolation and Screening of Endophytic Actinomycetes Producing Antibacterial Compound from Different Parts of Citrus aurantifolia. *Res J Pharm Technol*. 2008;1(2):112-115.
16. Geetali S. Ingawale and Anita S. Goswami-Giri. Isolation and characterization of bioactive molecule from Lantana camara. *asian J Res Chem*. 2014;7(3):339-344.
17. G. Jayanthy and S. Subramanian. Extraction, Isolation and Characterization of Rosmarinic Acid, a Major Polyphenol in Non-volatile Constituent of Mint Leaves. *asian J Res Chem*. 2013;6(12):1160-1165.
18. Ms. Shivani Watak DMSSP. Isolation of Apigenin From *Trigonellafoenum graecum* Seed Extract Using Column Chromatography on Lab Scale. *asian J Res Chem*. 2012;5(5):600-605.
19. Ambarsing P. Rajput MKP. Isolation and characterization of phytoconstituents from the chloroform extract of Abutilon indicumleaves (Family: Malvaceae). *asian J Res Chem*. 2012;5(11):1375-1380.
20. M. Khanahmadi FS and AA. Isolation and Structural Elucidation of Two Flavonoids from Ferulagoangulata (Schlecht) Boiss. *Asian J Res Chem*. 2011;4(11):1667-1670.
21. Mahajan REJ and RT. Isolation and Characterization of Endophytic Bacterial Flora from Some Indian Medicinal Plants. *asian J Res Chem*. 2011;4(2):296-300.
22. Krishna Veni Nagappan, Meyyanathan S N RBR and EK. A Liquid Chromatography Method for the Simultaneous Determination of Curcumin and Piperine in Food Products Using Diode Array Detection. *asian J Res Chem*. 2009;2(2):115-118.
23. Nikhat F, D. Satynarayanaand SE. Isolation, Characterization and Screening of Antioxidant Activity of the Roots of *Syzygium cuminii* (L) Skeel. *Asian J Res Chem*. 2009;2(2):218-221.
24. Battu PR, Reddy and M. Isolation of secondary metabolites from Pseudomonas fluorescens and its Characterization. *Asian J Res Chem*. 2009;2(1):26-29.
25. Safety F, Authority S, Health MOF, Welfare F, New Delhi. FSSAI Manual of methods of food analysis spices and condiments. 2012.
26. Hazra K, Council C, Sci A, Chowdary YA. UV-Visible Spectrophotometric Estimation of Curcumin in Nanoformulation. *Int J Pharmacogn*. 2015 ;2 (January):0-4. doi:10.13040/IJPSR.0975-8232.IJP.2(3).127-30.
27. Jasim F, Ali F. A novel method for the spectrophotometric determination of curcumin and its application to curcumin spices. *Microchem J*. 1988;38(1):106-110. doi:10.1016/0026-265X(88)90008-2.
28. Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. *Phytochem Anal*. 2009;20(4):314-319. doi:10.1002/pca.1129.
29. Boruah B. A study on the interactions of some synthetic and natural dyes of different charge types with surfactants and polymers. 2012;(11).
30. Dutta A, Boruah B, Saikia PM, Dutta RK. Stabilization of diketo tautomer of curcumin by premicellar cationic surfactants: A spectroscopic, tensiometric and TD-DFT study. *J Mol Liq*. 2013;187:350-358. doi:10.1016/j.molliq.2013.09.005.
31. Zetterström S. Isolation and synthesis of curcumin. 2012.
32. Berger S, Sicker D. Classics in Spectroscopy: Isolation and Structure Elucidation of Natural Products. Weinheim, Germany; 2009.