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ISOLATION AND ASSAY OF NANOCURCUMINOIDS

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ABSTRACT

The main of the present work was to develop an efficient method for extraction, formulation and evaluation of nano curcuminiods. In this study extraction of curcuminoids from turmeric was explored by various methods of extraction. For extraction soxhelet, ultrasonication and distillation methods were employed for separation of curcuminoids from turmeric powder under more or less varying same parameters. The results were analyzed and compared with reference method. The UV-Vis spectroscopy, HPLC and TLC were used for the confirmation and quantification of extract. The TLC analysis showed the three bands denoted the presence of three curcuminoids. The UV-Vis spectral analysis specified the absorption peak at 425nm. The HPLC studies implied the existence of three peaks for sonicated extract. The interpretation of the observations emphasized that the yield of extraction was higher for sonication method than remaining other extraction methods under varying parameters. The extracted sample was formulated into nano size particles. These were further evaluated for by UV, TLC, FTIR, SEM and bioassayed for antimicrobial and anti-inflammatory studies. These analytical studies confirmed the nano formulation and improved efficiency in its activity. The antimicrobial studies revealed the high activity against Gram–ve bacteria than Gram+ve bacteria. The anti-inflammatory studies suggested profound activity in comparison with diclofenac. Hence sonication method was considered as optimized extraction method which was time saving and less energy consuming favorable extraction method. The emphasized characteristics of improved extraction methods including cost-effectiveness (due to much saving in time and energy consumption) and environmentally benign nature make them more favourable extraction methods

INTRODUCTION

Turmeric is a flowering plant of the ginger family, Zingiberaceae, the roots of which are used in cooking. The bio-active polyphenol component of turmeric is curcumin, also known as diferuloylmethane (C21H20O6), with an ability to prevent and cure diseases Figure-1. Turmeric contains about 2-5% curcumin alone. Curcumin (diferuloylmethane renders its bright yellow colour to turmeric.

Curcuminoids exhibit a broad spectrum of biological and pharmacological activities including anti-oxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-parasitic, anti-mutagen, anti-cancer and detox properties [1-2.] It is known to be a good antioxidant, anti-inflammatory agent, anti-carcinogenic agent, anti-mutagenic agent, and anti-coagulant, widely used for combating a range of health

issues Figure 1.Commercial curcumin contains three main types of curcuminoids, i.e., curcumin (diferuloylmethane or "Curcumin I" about 77%), demethoxy curcumin ("Curcumin II" ∼17%) and bis demethoxy curcumin ("Curcumin III" ∼3%) Figure 2.Curcumin is a liposoluble compound and can be easily dissolved into an organic solvent such as methanol, ethanol, and acetone. However, poor water solubility often limits its biomedical uses using aqueous systems. This observation prompted us to examine turmeric extracts as a delivery system for curcumin and to examine the possibility of turmeric extract itself as candidate agent pharmacologic evaluation [1-2]. In this preliminary study, we used different solvents to extract the crude turmeric material and compared the curcumin concentrations in these extracts. Phytochemical analysis of the crude turmeric aqueous extract was used to identify key secondary metabolites. Furthermore, antioxidant assays were used to assess the bioactivity of purified curcuminoids. The present study describes screening of extraction method for isolation of curcuminoids from turmeric rhizome using non-polar solvents for extraction, isolation, identification and purification of curcuminoids followed by its analysis by HPLC, UV and TLC. The extracted curcuminoids were further reduced to nanosize in order to enhance their bioavailability. The formulated nanocurcuminoids were characterized by SEM, FTIR studies and bioassayed for their antimicrobial and anti-inflammatory activity.

MATERIALS AND METHODS:

Conventional extraction using soxhelet:

The turmeric powder was provided as a gift sample from department of pharmacognosy AGI. The soxhelet extraction as the reference method was performed as follows: 20g ground turmeric powder was weighed and embedded in a thimble and put in the soxhelet apparatus which was gradually filled with 150ml of acetone as the extraction solvent. The experiment was carried out at 56.63^{o} c within 6hrs. Acetone was separated using rota apparatus and the product i.e., curcuminoids dried and weighed. The dried powder was further used for analysis [3-7].

Ultrasound-assisted extraction of curcuminoids:

An ultra sonic bath (citizen scales) with tank capacity of 0.8 liter was used for extraction of curcuminoids. The bath power was 90W with 37KHZ frequency. For extraction 20g turmeric was dissolved in 150ml acetone and sonicated in ultrasonic bath. The extraction was performed at 25-30^{o}c for 30mins.The extract was filtered and dried[8].

Distillation method for extraction of curcuminoids:

20g ground turmeric powder was weighed and placed in to the distillation apparatus which was gradually filled with 150ml acetone as the extraction solvent. The experiment was carried at 56.63^{o}c within 30min. The product i.e., curcuminoids obtained upon drying was weighed[9].

Characterization of curcuminoids extracts:

High performance liquid chromatography (HPLC):

HPLC analysis of individual curcuminoids was performed according to Siregar et al. (2017) using Shimadzu LC-20AD (Kyoto, Japan) equipped with Rheodyne 7725i injection valve with a 20µL loop volume and binary gradient pump. Detection was carried out with Shimadzu Photodiode Array Detector (SPD-M20A) operated at a wavelength of 425 nm. Chromatographic separation was performed using Waters X-Bridge C-18 (250mm × 4.6mm i.d; 5µm), set at 45°C. The mobile phase used consisted of a binary mixture of acetonitrile-acetic acid 3.00% (49:51 v/v), was delivered in an isocratic manner with flow rate arranged at 1.08mL/min. For the preparation of a stock solution of samples, an accurately weighed amount of samples (about 200.0mg) was transferred into a 25mL volumetric flask, diluted with 10mL methanol, sonicated for 30 min, and then diluted with mobile phase to volume. The samples were homogenized and centrifuged for 10 min at 10,000 rpm. A portion of sample stock solution was diluted (1 in 20mL for CUR and 2.5 in 5mL for DMCUR) with mobile phase, mixed, and then filtered using 0.45µm filter before being

injected into HPLC system. Quantification of extracted curcuminoids was performed using the peak area at specific retention times and standard curve [11-12]. The amount of extracted curcuminoids (% w/w) from turmeric was calculated as follows:

$$\text{Curcumin yield (\%)} = \frac{M_{\text{extracted Curcumin}}}{M_{\text{pristine turmeric}}} \times 100$$

Thin layer chromatography (TLC) of the curcuminiods:

A glass plate layered with 2mm thickness of silica gel and activated at 105◦c for 15min after drying. Ethanolic solution of curcuminiods extract was spotted on this plate. Then the plate was run in a chromatographic beaker containing chloroform: Ethanol: Glacial acetic acid (9.4:0.5:0.1) as mobile phase[10].

Analysis of curcuminoids by UV-Vis Spectroscopy:

The extracted solutions were also analyzed for the presence of curcuminoids by UV-Vis spectrophotometer (Lab India UV-T60). An accurately weighed quantity of curcuminoids extracted samples of 10mg was dissolved in 10ml of ethanol which gave a solution of 10000ppi then from the prepared solution sample dilutions were made to prepare 100, 200, 300ppi solutions and checked for wavelength absorption in an amber colour volumetric flask. 1 ml of the above solution was diluted with ethanol to 10 ml in amber colour volumetric flask and the resulting solution was scanned for UV-Vis absorption using UV-Vis spectrophotometer in the range of 400 nm to 600 nm and λ-max was determined in Figure 6. Furthermore the spectrum of curcuminoids (λ-max) was compared with reported λ-max of the reference spectrum of curcuminoids [11-13].

Characterization of Nanocurcuminoids using Scanning electron microscope method (SEM):

SEM observation was performed on a SUPRA 60VP (Zeiss) electron microscope [37-38]. In this study, the accelerating voltage was set to 10 kV and the working distance was adjusted to around 3 mm. The contrast and brightness of the images were adjusted to optimal values so that particles could be easily distinguished from the background. The length scale within10 nm-60 nm range was calibrated with NIST RM 8011, 8012, and 8013. Using Smart SEM Software (Zeiss), image magnification could vary from 10 k times to 250 k times. For particle size analysis, the image processing software developed by National Institute of Health (NIH) was used(Image J Version 1.48v) and more than 200 particles were analyzed to extract the mean area-equivalent diameter (AED)and shape descriptors [14-16].

Characterization of Nanocurcuminoids Using FTIR:

An infrared spectrum of isolated curcuminoids was recorded using FTIR Spectrophotometer Shimadzu FTIR Germany (Happ-Genzel). The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about10t/in .The spectra's were recorded over the wave number of 8000 to 5001/cm. The scanning range was 500 to 4000 cm–1 and the IR spectra of samples were obtained using potassium bromide disc method[17-18]. Estimation of nanocurcuminoids content in turmeric rhizome was made using FTIR studies Figure 8.

Evaluation of Antimicrobial activity of Nanocurcuminoids by Agar well diffusion assay:

The antibacterial activity of the extract was determined by agar well diffusion method[39]. The overnight bacterial culture were diluted in the Mueller-Hinton broth to obtain a bacterial suspension of 108 CFU/ ml. Petri plates containing 20ml of Muller-Hinton broth Agar media were inoculated with 100µl of diluted cultures by pour plate technique and were allowed to dry in a sterile chamber. 5mm well was cut using a cork borer on the surface of the inoculated agar. The gel was loaded into wells and was allowed to dry completely. The antibacterial activity was assessed by measuring the inhibition zone [19].

Determination of MIC and Zone of Inhibition of Nanocurcuminoids:

A minimum inhibitory concentration is the lowest concentration of an antimicrobial that inhibit the growth of micro-organism after 18-24hrs. The samples were tested at different concentration. Sterile Muller Hinton A plates were prepared and 0.1 ml of the inoculums of test organism was added to it uniformly. Wells were prepared by using a sterile borer of diameter 5mm and nanocurcuminoids of different formulations is added to the wells in separate petriplates aseptically. The plates were incubated at 35-37ºC for 18-48 hours, a period of time sufficient for the growth. The zone of inhibition of microbial growth around the well was measured in mm. MIC was calculated from the fully grown plates. Record the size of the zone of inhibition against each cavity and the size is measured in mm with the help of scale or using antibiotic zone reader Figure 9 & 10.

Calculation:

L=3a+2b+c-e/5

H=3c+2d+c-a/5

Where

L=the calculated zone diameter for the lowest concentration of the standard curve

response line.

H=the calculated zone diameter for the highest concentration of the standard curve

response line.

C= average zone diameter of reference readings.

a,b,d,e = correct average value for the other standard solutions**.**

Evaluation of Anti Inflammatory Activity of Nanocurcuminoids: The Animals Wistar rats (100–150 g) were obtained from the Animal House, SANZYME Bio Labs Pvt. Ltd. Hyderabad-035. They were housed at a temperature of 24 ± 2°C, 12-hour light/dark cycles, 35–60% humidity, in polypropylene cages, and fed a standard rodent diet with water ad labium. Animals were deprived of food but not water 4 hours before the experiment. The drugs Diclofenac(API) (Reckitt Benckiser, Gurgaon, India), and Carrageenan (Sigma Chemicals, St. Louis, MO, USA) were procured from the respective companies and were used in the study[20-21].

Carrageenan-induced rat paw edema model:

The rats were divided into 4 groups (n = 6), each receiving distilled water (control), diclofenac 20 mg/kg p.o. (reference standard), and 2500 mg/kg (25 mg/kg) p.o. dose[2] of the Nano curcuminoids. Carrageenan (0.1 mL of 1%) was injected into the sub-plantar tissue of the right hind-paw of each rat. The volume of the carrageenan injected into the foot was measured at 0, 30, 60, 120, and 180 minutes using a plethysmometer (Biodevices, New Delhi, India). The percentage inhibition (PI) at each time interval was calculated:

$$PI = \frac{(V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{treated}}{(V_t - V_0)\ \text{control}} \times 100$$

V_0 = Mean paw volume at 0 hours

V_t = Mean paw volume at a particular time interval

RESULTS AND DISCUSSION:

Various extraction protocols for the separation of curcuminoids from turmeric were investigated. Even though the Soxhelet extraction was considered as the base method and the extraction yields were compared. Soxhelet extraction is one of the foremost and most common extraction techniques where in long extraction time at high temperature facilitate the extraction of target compound; moreover, the repeated contact of solvent with turmeric can enhance the extraction yield. The results of yield were confirmed by analytical techniques HPLC, UV, TLC, and FTIR. To confirm the presence of curcuminoids in the extracted sample, UV-Vis spectroscopy analysis was conducted using Lab India UV-T60. The absorbance spectra of standard and extracted curcuminoids using different methods were collected in the range of 300 to 600 nm as shown in Fig.6.

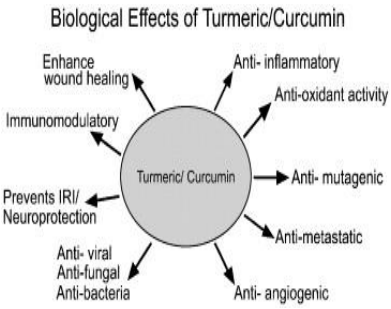


Figure 1: Biological effects of curcumin

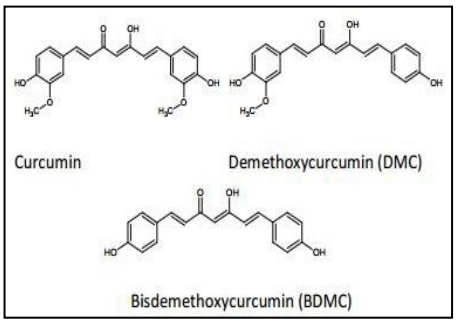


Figure 2. Types of Curcuminoids

HPLC Images of curcuminoids:

Distillation product 0.1mg:

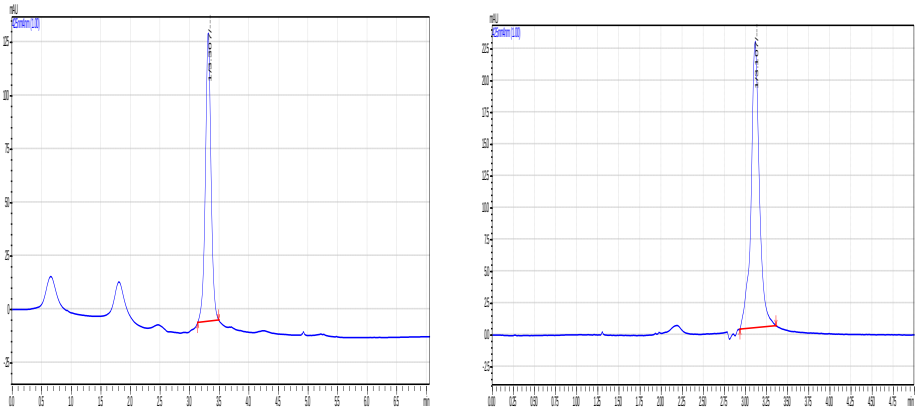
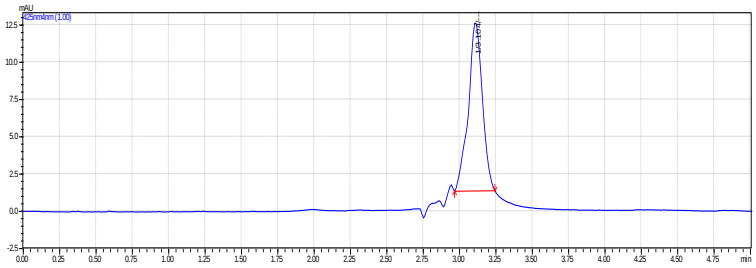
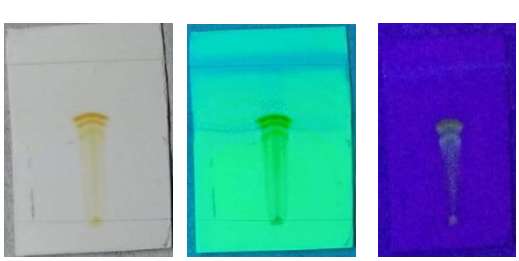


Figure 3 (a) Distillation method (b) Soxhelet method



(c) Sonication method

TLC for Standard Sample of Curcuminoids:



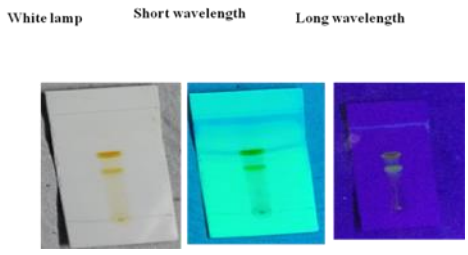
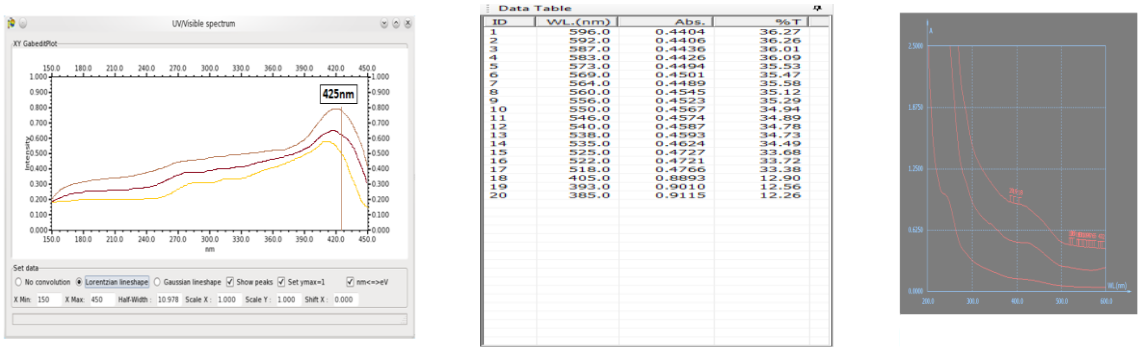


Figure 4: TLC images (a) Standard Sample (b) Isolated nanocurcumnoids



(a) **(b)** **(c)**

Figure 5 UV Analysis and UV spectra of sonicated Nanocurcuminoids extract (a) absorption spectra (b) Data table of UV analysis (c) absorption spectrum of different concentrations of sample

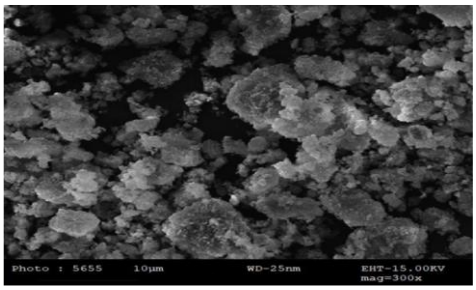


Figure 6: SEM image of Nanocurcuminoids

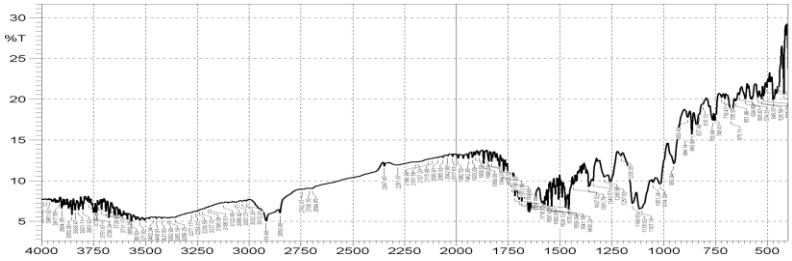


Figure 7 FTIR spectra of Nanocurcuminoids

Antimicrobial activity of Nanocurcuminoids:

Figure 8: Antibacterial activity (a) E.coli **(b) S.aureus**

Table 1: Antibacterial activity of Nanocurcuminoids

Components	Zone of inhibition of Escherichia.coli MTCC No: 2692 (in mm) Gram -ve		
Nanocurcuminoid	100 µl	200 µl	300 µl
	1.8	2.0	4.0
Components	**Zone of inhibition of Staphylococus aureus MTCC No: 902 (in mm) Gram +ve**		
Nanocurcuminoid	100 µl	200 µl	300 µl
	1.5	2.0	3.0

Table 2:Anti-inflammatory activity of Nanocurcuminiods:

Treatment	Paw volume in ml at different intervals (min) (Mean + S.E.M.)				
	0	30	60	120	180
Normal Control	0.1013± 0.0058	0.1012± 0.0058	0.1016± 0.0058	0.1017± 0.0058	0.1018± 0.0058
Inflammatory control	0.1205 ± 0.0079^{+++}	0.1806 ± 0.007^{+++}	0.2108 ± 0.0122^{+++}	0.2003 ± 0.0094^{+++}	0.1550 ± 0.0076^{+++}
diclofenac 20 mg/kg,p.o	0.1209 ± 0.0061	0.1407 ± 0.0071**	0.1309 ± 0.0054**	0.1402 ± 0.007**	0.1409 ± 0.0060
Nano Curcumin 2500mg/kg (25 mg) p.o. dose[2]	0.1016 ± 0.0070	0.132± 0.0057**	0.1458 ± 0.0042**	0.1442 ± 0.0071**	0.1482 ± 0.0136

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test.

+++ P<0.001 Vs Normal control & ** P< 0.01 Vs Inflammatory Control

The standard curcuminoids represented an absorption peak at around 425 nm. This characteristic peak which corresponds to the diarylheptanoid chromophore group of curcuminoids was observed in the spectrum of different samples obtained from Soxhelet, distilled, ultrasound-assisted extraction. Sonicated extract, soxhelet extract, distilled extract were analysed on spectrophotometer at 425nm with respect to standard graph. After concentrating each extract total yield was determined and percentage yield of curcuminoids were analysed by spectrophotometer at 425nm. From the above three extracts it was proved that sonicated method of extraction had given good yield. The absorbance of test solution measured at 425nm and the percentage of curcuminoids was calculated by calibration curve method. The method was validated for several parameters like linearity, accuracy. Calibration curve of curcuminoids was then plotted with absorbance on y-axis and curcuminoids concentration on x-axis Figure 5. HPLC analysis of the sonicated extract had showed the three characteristic peaks as that of standard Figure 3.The sonicated sample was run on TLC plate and three different spots of curcuminoids were obtained. After drying sonicated extract were weighed and weight percentage of curcumin, de-methoxycurcumin, and bis-demethoxy curcumin, RF values were calculated and found to be similar that of reported values. According to the RF values curcuminoids were analyzed by running standard curcuminiods along with the sample Figure 4. The functional groups were analysed using FTIR. The FTIR interpretation includes FTIR spectrum of Nanocurcuminoids formulations revealed distinct patterns of broad and sharp peaks ranging from 650 to 4000 cm−1. The appearance of distinctly sharp peaks at 648 and 1028 cm−1 depicted the presence of C-H bends and C-N stretches,

respectively. A strong peak at 1280.7 cm−1 indicated a C-H wag structure. The consecutive appearance of strong peaks at 1371.3 and 1431.1 cm−1 indicated stretching vibrations of C-H3 and C-C bonds, respectively. The appearance of broad peaks at 1633.6 cm−1 and a strong peak at 1587 cm−1 depicted stretching vibrations of C-C and N-H bend structures. A broad peak ranging from 2800 to 3000 cm−1 depicted the presence of H-C= O: C-H bends along with C-H stretches. Peaks in the range of 3500–3750 cm−1 appeared as characteristic for nano-curcuminoids, with strong peaks at 3631 and 3745 cm−1, indicating vibrations of O-H stretches in ring structures Figure 7. The SEM images showed berry grapes like circular concave shaped Nanocurcuminoids. The antimicrobial activity was performed by cup-plate method on Muller-Hinton Agar media. The zone of inhibition indicated that nanocurcuminoid formulation was highly active towards Gram-ve bacteria that Gram+ve bacteria Table 2. The two test organisms Escherichia coli MTCC No: 2692 and Staphylococcus aureus MTCC No: 902 Figure 8. The anti-inflammatory studies were done by carragenan method which indicated the mean paw volume inflammation was controlled by nano--curcuminoid formulation in comparison with diclofenac Table 2.

CONCLUSION:

Curcuminoids was extracted from turmeric through 3 methods - distillation, sonication and soxhelation. Obtained curcuminoids was further analysed by bio-analytical techniques. These were evaluated for higher yield of curcuminoids through HPLC. HPLC result of extraction of sample from 3 methods revealed that extract obtained from sonicated method had higher yield as compared with IP standard. In comparison to all the three methods of extraction sonication proved to be the best. Separation of curcuminoids from turmeric using number of advance methods was inspected and the outcomes were compared to those obtained from Soxhelet as the most common and reference method. Among these modern extraction methods ultrasound-assisted extraction show high extraction yields as high as Soxhelet method. Even the applications such as low extraction temperature, short extraction time and use of very few amount of solvent makes it more favourable extraction methods. In comparison to all methods of extraction sonication proved to be the best. Nano sized curcuminoid were formulated and evaluated through TLC, HPLC, UV, FTIR, and SEM and found that it matches with IP reference standards. Even bioassay of Nanocurcuminoids was carried using antimicrobial activity which shown that it was more active against Gram +ve bacteria (more zone of inhibition). It was also checked for anti-inflammatory activity. The analytical and bioassay evaluation studies indicated the improved efficiency in the activity of nano size curcuminoids.

REFERENCES:

1. Aggarwal BB, Surh, Shishodia S. The molecular target and therapeutic uses of curcumin in health and disease. Adv. Exper. Med. Bio. 2007: 595: 1-66.
2. Anand, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: Problems and Promises. Mol. Pharma. 2007: 4(6): 807–818. Anand P, Nair HB, Sung B. Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo. Biochem. Pharmacol. 2010:79(3):330-338. .
3. De castro, M. L. and Garcıa-Ayuso, L., Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chemica Acta 369: 1-10, 1998.
4. Duvoix, Blasius, R., Delhalle, S., Schnekenburger, M., Morceau, F., Henry, E., Dicato, M. and Diederich, M., Chemopreventive and therapeutic effects of curcumin, Cancer letters 223:181-190, 2005.
5. Esatbeyoglu, T., Huebbe, P., Ernst, I., Chin, D., Wagner, A. E. and Rimbach, G. Curcumin—from molecule to biological function. Angewandte Chemie International Edition 51:5308-5332, 2012.

6. Huang, X., Huang,X., Gong,Y., Xiao, H., Mcclements, D. J. and Hu, K., Enhancement of curcumin water dispersibility and antioxidant activity using core– shell protein–polysaccharide nanoparticles. Food Research International 87: 1-9, 2016.
7. Kurmudle, N., Kagliwal, L. D., Bankar, S. B. And Singhal, R. S. Enzyme-assisted extraction for enhanced yields of turmeric oleoresin and its constituents. Food Bioscience 3: 36-41, 2013.
8. Li, M., Ngadi, M. O. and Ma, Y., Optimisation of pulsed ultrasonic and microwave-assisted extraction for curcuminoids by response surface methodology and kinetic study. Food chemistry 165: 29-34, 2014.
9. Silva, L., Nelson, D. and Glória, M., Comparison of hydrodistillation methods for the deodorization of turmeric. Food Research International 38: 1087-1096, 2005
10. Pawar, H.; Gavasane, A.; Choudhary, P. A Novel and Simple Approach for Extraction and Isolation of Curcuminoids from Turmeric Rhizomes. Advances in Recycling & Waste Management 06, 2018.
11. Chauhan .Singh,and S.Agrawala Estimation of curcuminiods in curcuma longa by HPLC and spectrophotometric methods, Indian Journal of Pharmaceutical Sciences 61(1):58-601998.
12. Tiyaboonchai, Tungpradit, Plianbangchang "Formulation and characterization of curcuminoids loaded solid lipid nanoparticles". Int J Pharm. 06, 2007,337 (1–2): 299–306
13. Li,., Xiong, and Xu, Z. The formulation and delivery of curcumin with solid lipid nanoparticles for the treatment of on non-small cell lung cancer both in vitro and in vivo. Materials Science and Engineering *C*, 33: 4802-4808 2013.
14. Zabihi, F.; Xin, N.; Jia, J.; Cheng, T.; Zhao, Preparation Of Nano-Curcumin With Enhanced Dissolution Using Ultrasonic-Assisted Supercritical Anti-Solvent Technique. International Journal of Food Engineering 11(5) 2015.
15. Tomren, Másson, Loftsson, Tønnesen HH (June 2007). "Studies on curcumin and curcuminoids. Symmetric and asymmetric curcuminoids: stability, activity ". Int J Pharm. 338 (1–2): 27–34.
16. Curcumin Nanoformulation for Cervical Cancer Treatment Mohd S. Zaman[1], NeerajChauhan[1], Murali M.Yallapu[1], Rishi K.Gara[1], Diane M. Maher[2], Sonam Kumari[1], Mohammed Sikander[1], Sheema Khan[1], Nadeem Zafar[3], Meena Jaggi[1] & SubhashC. Chauhan[1], scientific reports. 2015 6:2005
17. Tiyaboonchai W, Tungpradit W, Plianbangchang P. "Formulation and characterization of curcuminoids loaded solid lipid nanoparticles". Int J Pharm. 06, 2007,337 (1–2): 299–306
18. Tajik, H., Tamaddonfard, E. and Hamzeh-Gooshchi, N., Interaction between curcumin and opioid system in the formalin test of rats. Pakistan Journal of Biological Sciences PJBS 10: 2583- 2586, 2007.
19. Tajik, Tamaddonfard, E. and Hamzeh-Gooshchi, N., The effect of curcumin (active substance of turmeric) on the acetic acid-induced visceral nociception in rats. Pakistan journal of biologicalsciences PJBS 11: 312-314, 2008.
20. Basniwal RK, Buttar SH, Jain KV. Curcuminoid nanoparticles preparation, characterization, and antimicrobial study. J Agric,Food Chem 2011;59:2056–61.
21. Jayaprakasha, Rao, Sakariah (2006). "Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin". Food Chemistry. 98 (4): 720–4.
22. Vaughn; Branum, A; Sivamani, RK (2016). "Effects of Turmeric (Curcuma longa) on Skin Health: A Systematic Review of the Clinical Evidence". Phytotherapy Research. 30(8): 1243–64.