Derivatization of Curcumin and the Effect of Resultant Derivatives on BRC-9 Breast Cancer Cells

Zivdar, Masumeh; Salahvarzi, Sabah*[+]

Department of Chemistry, Faculty of Science, Khorramabad Branch, Islamic Azad University, Khorramabad, I.R. IRAN

Dadgar, Zeynab

Department of biology, Faculty of Science, Arak University, Arak, I.R. IRAN

ABSTRACT: *Medicinal herbs have been taken into consideration for cancer treatment because of the high prevalence of cancer and the severe consequences of chemotherapy. Turmeric, the common name of the Curcuma longa plant, is one of these herbs as Indian spices being applicable for food spices as well as treatment of various diseases. The medicinal and biological effects of turmeric are actually associated with the main component of its rhizome, i.e. curcumin. Curcumin has antioxidant, antibacterial, anticancer, antifungal, and antiviral properties. In this research, curcumin derivatives were synthesized via a condensation reaction of some aromatic amines (aniline, 2-nitroaniline, 4-ethyl aniline) with curcumin. The effects of the molar ratio of amine: to curcumin on the type of products were also examined. The structure of the products was characterized by FT-IR, 1HNMR, and 13CNMR spectroscopy. The anticancer activities of compounds were investigated by the MTT assay (4,5-dimethylthiazol-2-yl) - 2,5 - diphenyltetrazoliu bromide test). It was evaluated with different dosages of curcumin derivatives at different times against BRC-9 breast cancer cells which exhibited the most potent anticancer activity.*

KEYWORDS*: Curcumin; Amine; Breast cancer; BRC-9 cell.*

INTRODUCTION

As the most challenging issue in global health and mortality, cancer is a disease in which cells begin uncontrollable growth in a part of the body [1-5]. Different types of cancer destroy the specific tissue of the body have their own particular features and treatment methods some commonly used ways are surgery, radiotherapy, chemotherapy, gene therapy, immunotherapy, inhibition of angiogenesis, and thermotherapy [6-11]. Most of these anticancer methods and medicines lead to acute consequences for the patient and might have toxic effects on healthy tissues [12]. Medicinal herbs, as a rich resource of antioxidants, have been surveyed to produce herbal anticancer medicines and investigate anticancer characteristics of which [13-19]. According to the world health organization, medicinal herbs would be the best resource for obtaining various medicines. For instance, curcumin a hydrophobic polyphenol is composed of a main aliphatic chain with the chemical name

***** *To whom correspondence should be addressed.*
+ *E-mail: s.salahvarzi@khoiau.ac.ir*
1021-9986/2022/4/1224-1231 8/$/5.08

of diferuloylmethane or 1,7-bis [4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione and molecular formula of $C_{21}H_{20}O_6$ as the principal component of turmeric [20-21]. Curcumin has been first explored almost two decades ago by Vogel isolated as an impure colored compound from the rhizome of the Curcuma longa plant [22]. Curcumin is confirmed as an antioxidant, anticancer, anti-Alzheimer, anti-Parkinson, anti-inflammatory, and anti-injury [23]. It extracted from the root of the turmeric plant contains 77% curcumin, 17% dimethoxy curcumin, and 3% bis methoxy curcumin [24]. Curcumin is soluble in ethanol, methanol, dimethyl sulfoxide, oil, and acetone, while insoluble in water and ether [25]. Solvent composition and solvent amount have a significant efficacy which are extensively investigated for extraction and purification of curcumin [26].

Curcumin has two phenolic rings which connected through the α,β-unsaturated systems. The molecular weight of curcumin is 368.38 g/mol having a melting point of 183ºC. Curcumin has stronger antioxidant activities in comparison to C and E vitamins [27]. Mechanisms that curcumin inhibits the formation of the tumor through which contain the combination of antioxidant, anti-inflammatory, anti-angiogenesis, anti-metastasic, proapoptotic, and cellular cycle inhibitory features, inducing inhibitory effects on cancer by regulating genes and molecules involved in these pathways [28]. Additionally, curcumin plays a significant role in various fields of cancer cells and particularly breast cancer cells. Breast cancer has been the most important cause of mortality between 40-55-year women in the world after lung cancer. The incidence of breast cancer in women aged above 50 years is about 70% [29]. Men may also catch breast cancer but its prevalence in women is 100 times men [30]. A wide variety of research has been performed in the field of curcumin derivatization and confirming the anticancer effects of this herbal material. *Nabati* and *Heidari* synthesized silylated derivatives of curcumin and suggested that bulky silylated substituents result in more stable compounds [31]. *Basil et al.* have synthesized two curcumin derivatives containing bis-dimethoxy curcumin (bDMC) and diacetyl curcumin (DAC) to improve the activity and stability of curcumin representing proper stability of derivatives relative to pure curcumin [32]. *Ding et al.* synthesized three groups of curcumin derivatives including phosphorylated, etherified, and esterified products to examine their anti-tumor activity against breast cancer (MCF-7), bone cancer cells (Hep-G2), cervical cancer cells (HeLa) [33]. *Lal et al.* examined one of the curcumin derivatives namely 3,4-dihydropyrimidinone/ thione [34]. Herein, some derivatives of curcumin were prepared from the reaction of some amines such as aniline, 2-nitroaniline, and 4-ethylaniline with curcumin in different molar ratios. The anticancer effects of obtained products were then examined on Breast Cancer Cells (BRC-9).

EXPERIMENTAL SECTION

Materials and devices

Curcumin, aniline, 2-nitroaniline, 4-ethylaniline, and methanol were purchased from Merck, Germany. The Bruker 400 (MHz) Nuclear Magnetic Resonance (NMR) device was used to identify the structure of the products, and the number and types of carbon and hydrogen. PerkinElmer RX I model by KBr tablet was used to identify product functional groups. The purity of the products and the reaction progress were measured by Thin-Layer Chromatography (TLC) and UV spectrophotometer. Silica gel or plate chromatography was used to separate and purify some products. A model sonication bath (Sonica) was used to dissolve the materials in the solvent and homogenize them. A model magnetic stirrer (Arec) was used to stir and heat the solutions. Sartorius TE313S digital scale weighing 0.001 decimal places was used to weigh the solids. A model centrifuge (Universal Pars Azma) was used to separate solid and liquid phases from suspension solutions.

To carry out this research, the BRC-9 breast cancer cell line was purchased from the Pasteur Institute, Iran. Cells were cultured in RPMI (Roswell Park Memorial Institute) medium (USA, Gibco) containing 10% Fetal bovine serum(FBS) (USA, ATCC (the American Type Culture Collection)), 2 mM L-glutamine solution, and penicillin-streptomycin (Gibco, Germany). The cells were plated in 25 cm^2 flasks and incubated at 37ºC with an atmosphere of 5% CO_2. Two days after culture initiation, the first medium replacement was performed, and then medium was changed two times per week till the bottom of the flask was covered with the cells (till confluency). The cells were trypsinized (trypsin-EDTA, Gibco, Germany) and passed to another culture flask as the first passage, and then the cultures were expanded through two additional subcultures which were used for further investigation.

Table 1: Comparison of melting point, efficiency, solubility, and color of products.

Reactants	Reaction conditions	Molar ratio	Melting point	Efficiency	solubility (in water)	Product color
Curcumin +Anilin	h 6 Ultrasonic	1:1	162 ° C	51.38 %	Good	Dark Orange Powder
		1:2	155 ° C	92.59 %		Red-orange powder
	h 12 Stirrer	1:1	165 ° C	32.87 %		Dark brick powder
		1:2	157 ° C	27.77 %		Dark red crystal
Curcumin +2-nitroanilin	h 6 Ultrasonic	1:1	200 ° C	24.59 %	Good	Dark Red Powder
		1:2	180 ° C	92.62 %		brick powder
	h 12 Stirrer	1:1	230 ° C	16.39 %		Dark brick powder
		1:2	200 ° C	16.40 %		Red powder
Curcumin +4-ethylanilin	h 6 Ultrasonic	1:1	167 ° C	63.82 %	Fair	brick powder
		1:2	164 ° C	29.78 %		brick powder
	h 12 Stirrer	1:1	170 ° C	28.93 %		Red-orange powder
		1:2	200 ° C	5.10 %		Red-brown powder

General procedure for the synthesis of curcumin derivatives

For the reaction of curcumin and several amines (Aniline, 2-nitroaniline, and 4-ethylaniline) with different stoichiometric molar ratios (1:1 and 1:2) in the presence and absence of an ultrasonic bath, the following procedures were utilized. The reactions were carried out using the typical method in two Erlenmeyer. 50 mL of curcumin (0.5 mmol, 0.18 g) was dissolved separately in methanol (15 mL) and then ultrasonicated (40kHz) for 1 h. After homogenization of the solution, the amine (0.5 and 1 mmol) was added dropwise to this solution at room temperature. One Erlenmeyer was ultrasonicated at 50ºC for 6 h and the other was stirred at 50ºC for 12 h. Both solutions remained at ambient temperature and the solvent was evaporated.

Treatment of cells with different derivatives of curcumin

In order to use these samples (curcumin derivatives), it is necessary to obtain an effective dose of curcumin in the cells. For that matter, Concentrations of 10, 20, and 40 μM were prepared from 7 different samples and added to the culture medium of the cells. For 24 hours, cells were exposed to different doses of curcumin derivatives. The MTT assay (methylthiazole tetrazolium) was then performed to evaluate cell proliferation.

Cell viability assays

The viability test on control and treated cells was carried out in a 96 well-plate using MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), where after 4 h of incubation, the mitochondrial succinate dehydrogenase in the living cells reduces the yellow color of tetrazolium into purple formazan. Then, 100 μL of DMSO was added to each well of the plate, and formazan crystals were dissolved at room temperature. The absorbance of solutions was measured on an automated microplate reader (SCO diagnostic, Germany) at 505 nm.

Statistical analysis

Statistical evaluation of the data was performed using the one-way analysis of variance (ANOVA) Tukey,s test, with the help of SPSS. Results were expressed as mean± S.D and $P< 0.05$ was accepted as the minimum level of significance

RESULTS AND DISCUSSION

The properties results (melting point, efficiency, solubility, and color) of all products for both methods were determined and presented in Table 1.

Chemical results

The curcumin was expected to undergo aza Michael's addition reaction with amine due to α,β-unsaturated structure of carbonyl (Fig. 1).

The results obtained from FT-IR, ^{1}HNMR, and ^{13}CNMR spectra revealed that an imine bond was obtained *via* a condensation reaction between the carbonyl group

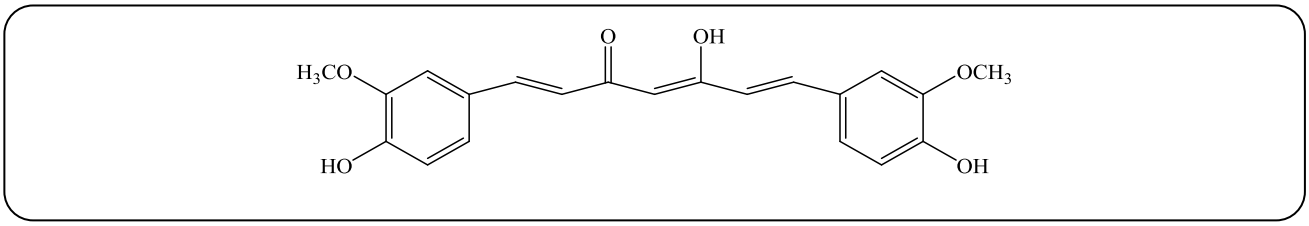

Fig. 1: Enol structure of curcumin.

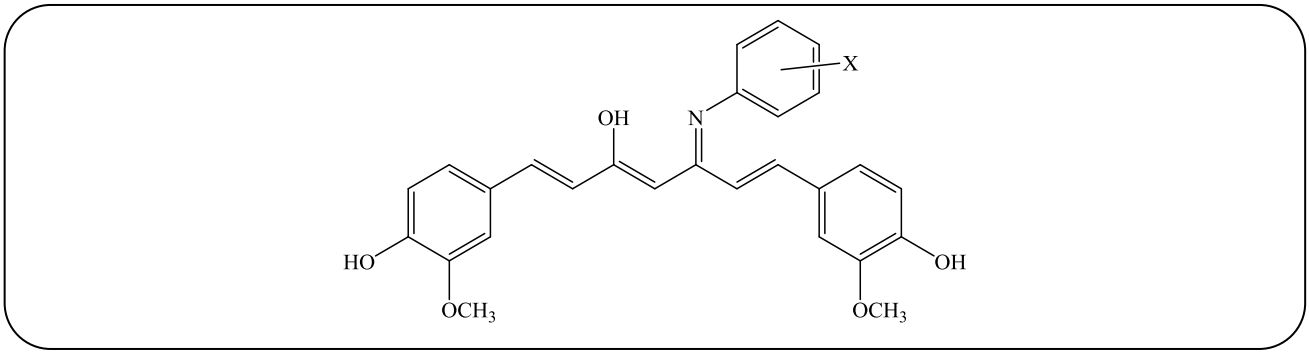

Fig. 2: Approved structure for derivatives product.

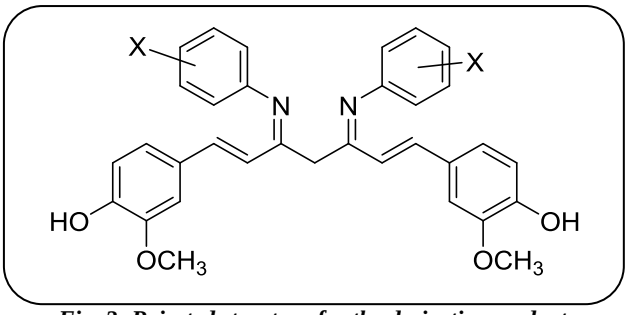

Fig. 3: Rejected structure for the derivative product.

of curcumin with NH_2 group of amine. This might be due to the tautomeric structures of curcumin. Curcumin will take an enol tautomeric structure because of the tendency to form conjugate bonds (Fig. 2).

Based on performed synthetic methods, no change was observed in the product structure by making a change in the curcumin-to-amine molar ratio from 1:1 to 2:1. This means that by doubling the amount of amine, only one carbonyl group interacts, and the other carbonyl converts to enolic OH group. Space congestion would be a cause in this case. The connection of the first mol of aromatic amine to the carbonyl of curcumin and the formation of the imine bond makes it difficult for the second mol of amine to approach another carbonyl group as a result of space congestion. The more influential reason was that by the formation of the first imine bond, the other carbonyl group converts to the enolic form to create a stable conjugate structure, so this stable structure does not tend to continue the reaction. In conclusion, the assumed structure for the product was rejected (Fig. 3) and the structure of the intended product was confirmed according to spectroscopic surveys (Fig.2).

The FT-IR, ^{13}CNMR, and ^{1}HNMR spectroscopy methods were used to determine the exact structure of the products. I n the FT-IR spectra for both molar ratios, the peaks above 3300 cm-1 are related to the tensile vibrations of the enoleic and phenolic OHs, respectively. Also, tensile vibrations of C = N and C = C appeared at 1626 cm $^{-1}$ and 1602 cm $^{-1}$, respectively. Dimethyl sulfoxide solvent was used for ^{13}CNMR and ^{1}HNMR spectroscopy. In ^{1}HNMR spectroscopy, the peak of the solvent is located at 2.5 ppm. In each peak spectrum, the hydrogen groups of the two methoxy groups appeared at about 3.8 ppm. The presence of enol and phenolic OHs in the ^{1}HNMR spectrum is also evident (Table 2). At ^{13}CNMR spectroscopy, the appearance of peaks at 40-39.3 ppm is related to solvent. The index peaks are listed in Table 2.

Effects of curcumin derivatives on the viability of cells

The results of SPSS statistical analysis showed that treatment of cells at 0 (control) and 10 μM of all samples after 24 hours, did not significantly change the number of cells. However, the title compounds showed the most potent activity with 20 μM against a number of cells. Also, the treatment of cells with 40 μM from different samples,

Table 2: The result of FT-IR, ^{13}CNMR, ^{1}HNMR spectroscopy.

Curcumin+Anilin 1:1 (Ultra)	FT-IR: 3342,3434 cm^{-1} (OH enolic, phenolic), 2830-3008 cm^{-1} (CH str) , 1626.43 cm^{-1} (C=N str), 1602.55 cm^{-1} (C=C Alken) , 1428-1510 cm^{-1} (C=C str), 1281.72 cm^{-1} (C-O-C). ^{1}HNMR: 9.741-9.785,7.149 ppm (OH), 5.149-7.578 ppm (H Aromatic), 6.747-6.843 ppm (H CH=C), 6.060 (H Active) , 3.845 ppm(H Methoxy groups). ^{13}CNMR: 56.13 ppm (C of OCH_3), 95.88 ppm(C of CH=C), 101.34 ppm (C Active), 183.68 ppm (Fourth Type enolic C).
Curcumin+Anilin 1:2 (Ultra)	FT-IR: 2838.51-3000 cm^{-1} (CH str), 1626.02 cm^{-1} (C=N str), 1585.60 cm^{-1} (C=C Alken), 1428.14-1511.20 cm^{-1} (C=C str). ^{1}HNMR: 3.854 ppm (H Methoxy groups), 6.066 ppm (H Active), 6.785-6.842 ppm (H of CH=C), 7.165-7.584 ppm (H Aromatic), 6.842,9.728 ppm (OH enolic,phenolic) ^{13}CNMR: 56.12ppm (C of OCH_3), 95.89 ppm (C of CH=C), 101.36 ppm (C Active), 183.68 ppm (Fourth Type enolic C).
Curcumin+Anilin 1:1 (Stirrer)	FT-IR: 2829-3025 cm^{-1} (CH str), 1626.79 cm^{-1} (C=N str),1602.50 cm^{-1} (C=C Alken), 1428.36-1510 cm^{-1} (C=C str), 1281.44 cm^{-1} (C-O-C). ^{1}HNMR: 3.749-3.852 ppm (H Methoxy groups), 6.065 ppm (H Active), 6.754-6.848 ppm (H of CH=C), 7.173-7.585 ppm (H Aromatic), 7.155,9.705 ppm (OH enolic,phenolic). ^{13}CNMR: 56.12 ppm (C of OCH_3), 95.88 ppm (C of CH=C), 101.35 ppm (C Active), 183.68 ppm (Fourth Type enolic C).
Curcumin+Anilin 1:2 (Stirrer)	FT-IR: 3294.11, 3361.34 cm^{-1} (OH enolic,phenolic), 2829-3031.9 cm^{-1} (CH str), 1622.37 cm^{-1} (C=N str), 1597.20 cm^{-1} (C=C Alken) , 1400-1506 cm^{-1} (C=C str), 1281.44 cm^{-1} (C-O-C).
Curcumin+2-nitroanilin 1:1 (Ultra)	FT-IR: 3392.24 cm^{-1} (OH enolic,phenolic), 2840-3008 cm^{-1} (CH str), 1625.17 cm^{-1} (C=N str), 1575.90 cm^{-1} (C=C Alken), 1429.48-1540.3 cm^{-1} (C=C str), 1384.70 cm^{-1} (NO_2). ^{1}HNMR: 3.84 ppm (H Methoxy groups), 6.061 ppm (H Active), 6.748-6.848 ppm (H of CH=C), 7.148-7.168 ppm (OH enolic). ^{13}CNMR: 56.12 ppm (C of OCH_3), 95.87 ppm (C CH=C), 101.33-101.42 ppm (C Active), 183.65 ppm (Fourth Type enolic C).
Curcumin+2-nitroanilin 1:2 (Ultra)	FT-IR: 3445.55 cm^{-1} , 3512.60 cm^{-1} (OH enolic,phenolic), 2834-3000 cm^{-1} (CH str), 1635.90 cm^{-1} (C=N str), 1541.25 cm^{-1} (C=C Alken) , 1454.54-1522 cm^{-1} (C=C str), 1370 cm^{-1} (NO_2).
Curcumin+2-nitroanilin 1:1 (Stirrer)	FT-IR: 3395.60-3602.20 cm^{-1} (OH enolic,phenolic), 2851-3008 cm^{-1} (CH str), 1625 cm^{-1} (C=N str), 1587.19 cm^{-1} (C=C Alken) , 1426.57-1508.47 cm^{-1} (C=C str)
Curcumin+2-nitroanilin 1:2 (Stirrer)	FT-IR: 3254.10-3394.95 cm^{-1} (OH enolic,phenolic), 2840-3014 cm^{-1} (CH str), 1624.37 cm^{-1} (C=N str), 1587.12 cm^{-1} (C=C Alken) , 1456.07-1513.20 cm^{-1} (C=C str), 1380 cm^{-1} (NO_2).
Curcumin+4-ethylanilin 1:1 (Ultra)	FT-IR: 3445 cm^{-1} (OH enolic,phenolic), 2851-2929.97 cm^{-1} (CH str), 1635.27 cm^{-1} (C=N str), 1588 cm^{-1} (C=C Alken) , 1420-1510 cm^{-1} (C=C str).
Curcumin+4-ethylanilin 1:2 (Ultra)	FT-IR: 3445.78 cm^{-1} (OH enolic,phenolic), 2934-3000 cm^{-1} (CH str), 1622.37 cm^{-1} (C=N str), 1541.25 cm^{-1} (C=C Alken) , 1420-1508.17 cm^{-1} (C=C str).
Curcumin+4-ethylanilin 1:1 (Stirrer)	FT-IR: 3260.50-3419.87 cm^{-1} (OH enolic,phenolic), 2834-3008 cm^{-1} (CH str), 1622 cm^{-1} (C=N str), 1541.25 cm^{-1} (C=C Alken) , 1451.74-1508.15 cm^{-1} (C=C str). ^{1}HNMR: 1.227-1.277 ppm (CH_2 ethyl), 0.867 ppm (CH_3 ethyl), 3.839 ppm (H Methoxy groups), 6.049 ppm (H Active), 7.015 ppm (OH enolic), 6.832-7.695 ppm (H Aromatic). ^{13}CNMR: 56.12 ppm (C of OCH_3), 95.86 ppm (C of CH=C), 101.35 ppm (C Active), 183.61 ppm (Fourth Type enolic C).
Curcumin+4-ethylanilin 1:2 (Stirrer)	FT-IR: 3271.70-3383.77 cm^{-1} (OH enolic,phenolic), 2931.02 cm^{-1} (CH str), 1624.25 cm^{-1} (C=N str), 1587.25 cm^{-1} (C=C Alken), 1455.99-1508.71 cm^{-1} (C=C str).

after 24 hours, resulting in cell death of approximately 50% of the cells and the number of cells was halved, compared to the control group (0 μM). Therefore, the aniline-curcumin derivatives have no significant difference in the excretion of cancer cells (BRC-9 cell line) compared to pure curcumin. This means that the dose of 20 and 40 μM curcumin and its derivatives (regardless of their manufacturing method), reduced the growth and proliferation of these cells in the 24 hours treatment of breast cancer cells (BRC-9) compared with the control group (Table 3).

Several studies have been performed on the potential performances of curcumin in animals, but many reports determine multiple activities of curcumin, the use of this material has not been confirmed to treat human diseases. Accordingly, the consumption of this medicine for humans has been safe on a little scale. This natural product can regulate different signaling paths and affect numerous molecular targets. Since curcumin is safe due to nontoxicity, its low biostability has limited its use as a therapeutic agent. Therefore, various strategies are developed to improve its biostability. However, low cost, pharmacological safety,

Table 3: Cell viability assay, Comparison of the number of cells after treatment with different doses of 6 samples, after 24 hours, using MTT test. Values are means ±SD. The meanings of the different code are different, All values are expressed as the mean ±SD. a,b,c,d: means which are significantly different from each other (one-way ANOVA, Tukey,s test, P< 0.05).

Dose(Micromolar) Samples	0	10		40
S1 (curcumin:aniline,1:1,ultra)	2029 [a] ±2.6	2039 [a] ±1.2	883 [b] ±5.1	614 [c] ±7.7
S2 (curcumin:aniline,1:2,stirrer)	2002 [a] ±7.3	2092 [a] ±8.3	853 [b] ±4.4	543 [c] ±6.3
S3 (curcumin:aniline,1:1,stirrer)	2131 [a] ±3.1	1951 [a] ±6.4	769 [b] ±4.2	483 [c] ±3.3
S4 (curcumin:aniline,1:2,ultra)	2039 [a] ±2.2	2097 [a] ±1.1	815 [b] ±2.1	491 [c] ±1.6
S5(curcumin:4-ethylaniline,1:1,ultra)	2011 [a] ±6.1	1899 [a] ±4.1	759 [b] ±4.4	589 [c] ±3.2
Curcumin	1997 [a] ±3.2	1887 [a] ±3.2	761 [b] ±1.3	570 [c] ±4.8

efficacy and different molecular targets have made curcumin a promising product to prevent and treat human diseases. According to various research studies, curcumin has been proven to be able to treat most diseases, such as cancer, while its poor water solubility restricts its clinical application for cancer therapy. The medicine should be water-soluble to flow inside the bloodstream. In spite of field research for decades, the development of an effective method to transfer poor water-soluble curcumin to cancer cells had been a challenge. Recently, some researchers from the University of Illinois and the University of Utah, USA, have succeeded to find a novel route. Supervised by Dipanjan Pan from the University of Illinois, these researchers can insert curcumin into tumors and destroy cancer cells by increasing their solubility [35]. Thus, a combination of curcumin with other compounds would increase its solubility and permeation to cancer cells. The aim of this study was to synthesize derivatives of curcumin by the interaction of some kinds of amine with curcumin in order to increase the solubility and biocompatibility of this molecule as well as to assess its anticancer effects on BRC-9 breast cancer cells. Obtained results represented a significant decrease in the number of cells after treatment with 20 μM dosage. Also, the treatment of cancer cells with 40 μM dosage in different samples has caused cellular death by almost 50% after 24 h so the number of cancer cells reached almost half relative to the control group (0 μM). Hence, aniline-curcumin-derivatives have no significant difference in applying lethal characteristics to cancer cells in comparison to pure curcumin. It is apparent that the growth and reproduction of BRC-9 breast cancer cells have been reduced with 20 and 40 μM dosage of aniline- derivative and curcumin.

The examination of the effects of sonication on the particle's size, melting point, and efficacy of products was done. It has been reported that the ultrasonic method has important effects on the mean particle size and the melting point of products. This is along with the decrease in melting point in all products with respect to free sonication.

It is important to note that this synthetic protocol is cost-effective in addition to other advantages such as reasonably soluble derivatives with fair to good solubility in water at room temperature in comparison with insoluble curcumin. Also, the efficacy of products was noticeable for the ultrasonic bath method when compared with the stirrer method (Table 1). In this regard, the Specific chemical structure and multiple forces of curcumin made it possible for this molecule to participate in most physical, chemical, and biological reactions. Rings of curcumin have made a hydrophobic media. Furthermore, its tautomeric structure has the main effect on the hydrophobicity and polarity of the molecule. This has made it to be applied to other kinds of cancer.

CONCLUSIONS

Derivatives of curcumin were synthesized from the reaction of aromatic amines, such as aniline, 2-nitroaniline, and 4-ethyl aniline with curcumin by the controlled ultrasonic method in this research. The effects of the molar ratio of amine: curcumin (1:1 and 1:2) on the products were investigated. It has been reported that the stoichiometric ratio has a significant improvement in relation to the type of products. The structures of all compounds were then characterized by FT-IR, ^{1}HNMR, and ^{13}CNMR spectroscopy. The comparison of derivatives of curcumin with curcumin has revealed that appropriate amines are able to form proper structure with curcumin, therefore curcumin derivatives

curcumin derivatives showed better water solubility in comparison with curcumin. This property makes these derivatives suitable for acting as an effective anticancer compound. Thus, the MTT assay (methylthiazole tetrazolium) has been sufficiently investigated to evaluate cell proliferation (BRC-9 breast cancer cells). The results clearly demonstrated that compounds have significant potential to be applied to various kinds of cancer.

Supplementary Information

The NMR and FT-IR data for the structural analyses of compounds have been deposited with the Shahid Beheshti University. Copies of this information are available on request in a separate file.

Acknowledgments

The authors would like to appreciate the Islamic Azad University, Khorramabad branch (Iran) for supporting this research.

Received : Apr. 26, 2021 ; Accepted : May 17, 2021

REFERENCES

[1] Serrano A., Ros G., Nieto G., Bioactive Compounds and Extracts from Traditional Herbs and Their Potential Anti-Inflammatory Health Effects. *Medicines*, **5:** 76-85(2018).

[2] Siegel RL., Miller KD., Jemal A., Cancer Statistics *CA. Cancer. J. Clin*, **69:** 7-34(2019).

[3] Pishgar F., Ebrahimi H., Saeedi Moghaddam S., Fitzmaurice C ., Amini E., Global, Regional and National Burden of Prostate Cancer, 1990 to 2015: Results from the Global Burden of Disease Study 2015. *J. Urol*, **199:** 1224-32(2018).

[4] Mcaloon CJ., Boylan LM., Hamborg T., Hamborg T., Stallard N., Osman F., Lim P B, Hayat S A., The Changing Face of Cardiovascular Disease 2000-2012: an Analysis of the World Health Organisation Global Health Estimates Data, *Int. J. Cardiol*, **224:** 256-64(2016).

[5] Maruthappu M., Watkins J., Noor A.M., Williams C., Ali R., Sullivan R., Zeltner T., Atun R., Economic Downturns, Universal Health Coverage, and Cancer Mortality in High-Income and Middle-Income Countries, 1990-2010: A Longitudinal Analysis, *The Lancet*, **388(10045):** 684-95 (2016).

[6] Johnston WW., Cytologic Diagnosis of Lung Cancer. Principles and Problems, *Path. Rese.Prac*, **181:** 1-36 (1986).

[7] Sikora E., Bielak-Zmijewska A., Mosieniak G., Targeting Normal and Cancer Senescent Cells as a Strategy of Senotherapy, *Ageing. Res. Rev*, **55:** 100941 (2019).

[8] Chávez J.P., Gürbüz B., Pinto C.M.A., The Effect of Aggressive Chemotherapy in a Model for HIV/AIDS-Cancer Dynamics, *Comm. Nonlin. Scie. Nume. Simul*, **75:** 109-20 (2019).

[9] Lachance J.C., Radhakrishnan S., Madiwale G., Guerrier S., Vanamala J.K.P., Targeting Hallmarks of Cancer with a Food System-based Approach, *Nutrition*, **69:** 110563 (2019).

[10] Blaes A., Prizment A., Koene RJ., Konety S., Cardio-oncology Related to Heart Failure: Common Risk Factors Between Cancer and Cardiovascular Disease, *Heart. Fail. Clin.*, **13:** 367-380 (2017).

[11] Riscal R., Skuli N., Simon MC., Even Cancer Cells Watch their Cholesterol, *Mol. Cell.* **76:** 220-31 (2019).

[12] Zhou M., Zhang X., Yu C., Nan X., Chen X., Zhang X., Shape Regulated Anticancer Activities and Systematic Toxicities of Drug Nanocrystals *in Vivo*, *Nanomedicine*, **12:** 181-9 (2016).

[13] Agarwal N., Majee C., Chakraborthy G.S., Natural Herbs as Anticancer Drugs, *Inter. J. Pharm.Tech. Res.*, **4:** 1142-53 (2012).

[14] Guldiken B., Ozkan G., Catalkaya G., Ceylan F.D., Ekin Yalcinkaya I., Capanoglu E., Phytochemicals of Herbs and Spices: Health Versus Toxicological Effects, *Food. Chem. Tox*, **119:** 37-49 (2018).

[15] Amjad S., Jafri A., Sharma A.K., Serajuddin M., A Novel Strategy of Nanosized Herbal Drugs and their Delivery in the Treatment of Diabetes: Present Status and Future Prospects. *J. Herb. Medic.* In press, Corrected Proof, 100279 (2019).

[16] Harwansh R.K., Deshmukh R., Rahman M.A., Nanoemulsion: Promising Nanocarrier System for Delivery of Herbal Bioactives, *J. Drug. Deli. Sci. Tech.*, **51:** 224-33 (2019).

[17] Dadashpour M., Firouzi-Amandi A., Pourhassan-Moghaddam M., Maleki M.J., Soozangar N., Jeddi F., Nouri M., Zarghami N., Pilehvar-Soltanahmadi Y., Biomimetic Synthesis of Silver Nanoparticles Using Matricaria Chamomilla Extract and their Potential Anticancer Activity Against Human Lung Cancer Cells, *Mater. Sci. Eng. C Mater. Biol. Appl*, **92:** 902-12(2018).

[18] Rocha-Guzmán N.E., González-Laredo R.F., Vázquez-Cabral B.D., Moreno-Jiménez M.R., Gallegos-Infante J.A., Gamboa-Gómez C.I.., Flores-Rueda A.G., Oak Leaves as a New Potential Source for Functional Beverages: Their Antioxidant Capacity and Monomer Flavonoid Composition. *Funct. Medic. Beve*, 381-411 (2019).

[19] Mansoori B., Mohammadi A., Amin Doustvandi M., Mohammadnejad F., Kamari F., Gjerstorff M.F., Baradaran B., Hamblin M.R., Photodynamic Therapy for Cancer: Role of Natural Products, *Photo. Photodyn. Ther.*, **26:** 395-404 (2019).

[20] Xu G.W.J., Si G., Wang M., Cheng H., Chen B., Zhou S., Preparation., Photoluminescence Properties and Application for *in Vivo* Tumor Imaging of Curcumin Derivative-Functionalized Graphene Oxide Composite, *Dyes. Pigment*, **141:** 470-8 (2017).

[21] Sheikh E.B.M., Tripathi M., Role of Nano-Curcumin: A Treatment for Cancer. *J. Medic. Plant. Stud*, **5:** 394-7 (2017).

[22] Vogel A.P.J., Examen Chimique de La Racine de Curcuma. *J. Pharm.*, **1:** 289-300 (1815).

[23] Ravindran J., Prasad S., Aggarwal B.B., Curcumin and Cancer Cells: How Many Ways Can Curry Kill Tumor Cells Selectively? *AAPS. J.*, **11:** 495-510 (2009).

[24] Wilken R., Veena M.S., Wang M.B., Srivatsan E.S., Curcumin: A Review of Anti-Cancer Properties and Therapeutic Activity in Head and Neck Squamous Cell Carcinoma, *Mol. Cancer*, **10:** 12 (2011).

[25] Tonnesen H.H., Karlsen J., Studies on Curcumin and Curcuminoids. VI. Kinetics of Curcumin Degradation in Aqueous Solution, *Z. Lebensm. Unter. Forsch.*, **180:** 402-4 (1985).

[26] Doosthosseini H., Salehi Z., Rezaei M., Ghelich P., Optimized Method for Curcumin Separation from Turmeric Oleoresin. *Iran. J. Chem. Chem. Eng. (IJCCE)*, **38**: 141-148 (2019).

[27] Toda S., Miyase T., Arichi H., Tanizawa H., Takino Y., Natural Antioxidants. III. Antioxidative Components Isolated from Rhizome of Curcuma Longa L, *Chem. Pharm. Bull. (Tokyo)*, **33:** 1725-1728 (1985).

[28] Sarkar FH., Li Y., Wang Z., Padhye S., Lesson Learned From Nature for the Development of Novel Anti-Cancer Agents: Implication of Isoflavone, Curcumin, and their Synthetic Analogs, *Curr. Pharm. Des.* **16:** 1801-1812 (2010).

[29] Desantis C.E., Ma J., Gaudet MM., Newman L.A., Miller K.D., Goding Sauer A., Jemal A., Siegel R L., Breast Cancer Statistics, 2019, *CA. Cancer. J. Clin*, **69:** 211-33 (2019).

[30] Sadjadi A., Nouraie M., Ghorbani A., Alimohammadian M., Malekzadeh R., Epidemiology of Breast Cancer in the Islamic Republic of Iran: First Results from a Population-Based Cancer Registry, *East. Mediter. Health. J*, **15:** 1426-1431 (2009).

[31] Nabati M., Heidari H., Isolation and Characterization of Curcumin from Powdered Rhizomes of Turmeric Plant Marketed in Maragheh City of Iran with Soxhlet Technique, *Iran. Chem. Commun.*, **2:** 236-43 (2014).

[32] Basile V.F.E., Lazzari S., Belluti S., Pignedoli F., Imbriano C., Curcumin Derivatives: Molecular Basis of their Anti-Cancer Activity, *Biochem. Pharmacol.* **78:** 1305-15 (2009).

[33] Ding L., Ma S., Lou H., Sun L., Ji M., Synthesis and Biological Evaluation of Curcumin Derivatives with Water-Soluble Groups as Potential Antitumor Agents: an in Vitro Investigation Using Tumor Cell Lines, *Molecules*, **20:** 21501-14(2015).

[34] Lal J., Gupta SK., Thavaselvam D., Agarwal DD., Synthesis and Pharmacological Activity Evaluation of Curcumin Derivatives, *Chin. Chem. Lett*, **27:** 1067-72 (2016).

[35] Datta S., K. Misra S., Lal Saha M., Lahiri N., Louie J., Pan D., J. Stang P., Orthogonal Self-Assembly of an Organoplatinum(II) Metallacycle and Cucurbit[8]uril that Delivers Curcumin to Cancer Cells, *Proce. Nati. Acad. Scien*, **115 (32):** 8087-8092 (2018).