



# Synergistic Effect of *Curcuma longa* Extract in Combination with *Phyllanthus niruri* Extract in Regulating Annexin A2, Epidermal Growth Factor Receptor, Matrix Metalloproteinases, and Pyruvate Kinase M1/2 Signaling Pathway on Breast Cancer Stem Cell

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## Abstract

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**AIM:** This study aimed to investigate the synergistic effects of the combination between *Curcuma longa* extract (CL) and *Phyllanthus niruri* extract (PN) in inhibiting optimally the MDA-MB-231 breast cancer stem cells (BCSCs) growth and metastatic by exploring the target and molecular mechanism using integrative bioinformatics approaches and *in vitro*.

**METHODS:** CL and PN extracts were prepared by maceration method using ethanol 70%. The antiproliferative effect of CL and PN single and combination treatment was examined by 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide assay. The bioinformatic approach was performed to identify molecular targets, key proteins, and molecular mechanism of curcumin and phyllanthin as CL and PN secondary metabolite, respectively, targeted at stemness and migration pathway of BCSCs.

**RESULTS:** The *in vitro* study showed that CL and PN possess cytotoxic activity in time- and dose-dependent manner. The combination of CL and PN has a synergistic effect by modulating the sensitivity of cells. Using a bioinformatics approach, the annexin A2 (ANXA2), epidermal growth factor receptor (EGFR), matrix metalloproteinases (MMPs), and pyruvate kinase M1/2 (PKM) as potential targets of curcumin and phyllanthin correlated with metastatic inhibition of BC. In addition, molecular docking showed that curcumin and phyllanthin performed similar or better interaction to stemness differentiation regulator pathway particularly histone deacetylase 1, EGFR, Heat Shock Protein 90 Alpha Family Class B Member 1, Hypoxia Inducible Factor 1 Subunit Alpha, and MMP9.

**CONCLUSION:** Combination of CL and PN has potential for the treatment of metastatic BCSCs by targeting ANXA2, EGFR, MMPs, and PKM to resolve stemness and inhibit of BCSCs.

## Background

Breast cancer (BC) is the most common cancer among women, presenting a major public issue around the world [1]. BC stem cells (BCSCs), a subpopulation of cancer cells that have tumor-initiating properties play a critical role in cancer recurrence, stemness, and metastasis [2], [3]. The highest mortality rate of BC is caused by a cancer recurrence and metastatic complication [4]. The previous study reported that the activation of the epidermal growth factor receptor (EGFR) signaling induces epithelial-mesenchymal transition (EMT) correlated with aggressive metastatic features [5]. Recent studies provided evidences that annexin A2 (ANXA2) is involved in EGFR signaling.

The ANXA2 expression also may promotes the TGF- $\beta$  upregulation leading to activation of matrix metalloproteinases (MMPs). This phenomenon correlated to metastatic enhancement and stemness phenotype of BCSCs [6]. Therefore, blocking ANXA2 function lead to the inhibition of the EGFR and TGF- $\beta$  downstream pathway associated with the reduce of BCSCs stemness ability in cell progression, migration, and metastatic reduction [7]. On the other hand, the pyruvate kinase M1/2 (PKM2) overexpression, promotes metastasis of cancer cells that correlated with ANXA2 expression *in vivo* [8]. In recent years, increased attention has focused on ANXA2 and its role in regulating BC development [9], [10].

In well-established medical treatment, the use of chemotherapy to control metastatic BC

has been developed as a therapeutic protocol of cancer, irrespective of surgical treatments [11], [12]. Unfortunately, the chemotherapy effectiveness is limited by acquired chemoresistance. The previous study reported that overexpression of ANXA2 associated with chemoresistance evidence [13]. Therefore, developing natural chemotherapeutic agents to eliminate cancer cells without emerging drug resistances remain still a challenging project. The benefit used of plant extracts with natural multi-targeting capability in killing cancer cells and relatively safer to normal tissues is the one predominance reason to utilize the medical plant extracts including *Curcuma longa* (CL) and *Phyllanthus niruri* (PN) [14], [15], [16].

CL and PN are the common medicinal plants used in Indonesia with many biological effects including anticancer activity on various cancer cells [17], [18], [19], [20], [21]. Recent studies have shown that CL extracts might suppress cancer cell proliferation by inducing the G0/G1 cell cycle arrest and trigger cell apoptosis [22], [23], [24]. Furthermore, curcumin as one of the secondary metabolite compound of CL extracts has specific-binding with ANXA2 proteins to reduce the growth and size of tumor mass [18], [25], [26]. On the other hand, several studies reported that PN extracts also possess cytotoxic effects on various cancer cells such as promyelocytic leukemia HL-60 cell lines, human oral cancer HSC and BC [20], [27]. The phytochemical studies of the PN have shown that those extracts contain a variety of components, particularly phyllanthin inhibited metastatic of lung cancer through decrease MMP9 expression [28].

Recently, the combination of two or more natural chemotherapeutic agent might achieve efficacy with lower doses and in the absence of toxicity. Therefore, using the combination CL and PN was supposed to have synergistic effects in optimally inhibiting the cancer cells growth and possibly fewer side effects. In this study, we use MDA-MB-231 cells, a highly metastatic BC cell line with a high population of BCSCs. This study aimed to investigate the synergistic effects of the combination between CL and PN in inhibiting optimally the MDA-MB-231 BC cells growth and metastatic by exploring the target and molecular mechanism using integrative bioinformatics approaches.

## Materials and Methods

### Plant material

CL and PN were collected in November 2019 and February 2020, respectively, in Tawangmangu, Karanganyar Central Java, Indonesia (Latitude 7°40'39.3"S; Longitude 111°08'09.4"E). The plant identification was verified by the biologist from the

center for research and development of medicinal plants and traditional medicine (B2P2TOOT), Indonesia. For biological determination, the herbs of PN and rhizome of CL were dried with circulated at 40°C and renewal of air oven until completely dehydrated.

### Extraction procedure

CL and PN were cleaned and air-dried to constant weight at room temperature for 3 days before being ground powder in a blender. The powder of PN (500 g) and CL (500 g) was extracted individually by maceration method using ethanol for 72 h (three cycles) based on Tanvir *et al.* [29] with slight modification. Then, the solutions were filtered through Whatman no.1 filter paper and the solvent was evaporated under reduced pressure (100 psi) in a rotary vacuum evaporator (IKA HB 10 basic) at 40°C to result in the crude extracts. The extracts were collected and preserved at 4°C for subsequent analysis.

### Cell culture

MDA-MB-231 (ECACC #92020424) was maintained in Dulbecco's Modified Eagle's Medium-high glucose (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 12.5 µg/ml Amphotericin B (Gibco, USA), 150 µg/ml Streptomycin, and 150 IU/ml Penicillin (Gibco, USA). Cells were cultivated at 37°C under 5% CO<sub>2</sub>. Culture media were renewed every 2 to 3 days, and cells were subculture when confluent of 80–90%. For assays, only cells with >90% viability, passage number <10, and in the log growth phase were used according to Amalina *et al.* [30].

### Cytotoxic assay

The cytotoxic assay was based on a 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to Mosmann [31] with slight modification. Briefly, the density of  $5 \times 10^3$  cells/well was seeded into 96 well-plate and incubated at 37°C under 5% CO<sub>2</sub> for 24 h. Subsequently, cells were treated in a triple with CL rhizome (5–200 µg/ml) and PN herbs (5–200 µg/ml) and exposed for 24 h. Untreated cells were regarded as negative controls. After treatment, cells were treated with 0.5 mg/mL of MTT (Biovision) and incubated further for 4 h. MTT formazan was soluble using 100 µl DMSO and incubate for 15 min. After incubation, the absorbance was measured by ELISA reader (BioRad iMark™ Microplate Reader) at λ 595 nm. The absorbance was transformed into a percentage of cell viability by comparing the treated group with the untreated group at a particular time course. To calculate IC<sub>50</sub> value, linear regression between concentration [x] and % cell viability [y], giving the equation  $y = Bx + A$ . Using the linear equation of this

graph for  $y=50$  value  $x$  point becomes  $IC_{50}$  value, that is the concentration that prevents the cell proliferation of 50%. The data of this study were carried out with three replication experiments [32].

### Combination activity

The first set of combination experiment, we evaluate the effectiveness of a particular concentration of CL rhizome, PN herbs, and its combination on MDA-MB-231 cells, was determined using MTT assay as described previously. MDA-MB-231 cells ( $5 \times 10^3$ ) were seeded in 96-well microplate and incubated at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  for 24 h. The cells were treated with one-half  $IC_{50}$ , one-fourth  $IC_{50}$ , one-eighth  $IC_{50}$ , and one-sixteenth  $IC_{50}$  of CL, PN, and its combination for 24 h. After 24 h of treatment, the percentage of viable cells was determined using the *in vitro* cytotoxicity assay method described above.

### Analysis of combination activity

The combination of CL and PN was determined using isobologram analysis and represented with the combination index value. CI value  $<1$  indicated the synergism effect and CI value  $>1$  indicated antagonism. The combination index analysis was based on the principle of the median effect and calculated using the following formula. Combination index =  $D_1/[Dx]_1 + D_2/[Dx]_2$ , where  $D_1$  and  $[Dx]_1$  are concentrations of CL Rhizome and PN herbs, respectively, which inhibit cell growth to 50% of control when used alone, and  $D_2$  and  $[Dx]_2$  are concentrations of CL rhizome and PN herbs, respectively, which have the same effect when used in combination [33]. Combination index values based on the Chou-Talalay method, calculated using CompuSyn software, indicate the effects of drug combinations [34], [35].

### Data collection and processing

Cytotoxicity and mRNA arrays data were collected from NCI 60 direct target proteins (DTP) website. The analysis of COMPARE from the NCI 60 cell line panel was employed using public library procedures to collect drugs compounds that have similarities with Curcumin and Phyllanthin. The similarity of the pattern is expressed as a Pearson coefficient of correlation. The list of compounds and genes in this study was limited to the Pearson correlation  $<-0.5$  and  $>0.5$ .

### Analysis of functional and pathway enrichment

Analysis of Gene Ontology (GO) and Kyoto encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed by the Database

for Annotation Visualization and Integrated Discovery, the cutoff value was selected with  $p < 0.05$ . In addition, pathway enrichment was also performed using Overrepresentation Enrichment Analysis (ORA) from WEB-based GENE SeT Analysis Toolkit (WebGenestalt) with FDR  $<0.05$  as the cutoff value.

### Construction of protein-protein interaction (PPI) network

STRING-DB v11.0 was used to construct the PPI network. Confidence scores  $>0.4$  were considered to be significant. Cytoscape software was used to visualize the PPI network. 10 Genes with the highest degree of the score were evaluated using Cytohubba and selected as hub genes.

### Molecular docking

Simulation of docking was conducted to predict the binding interaction of curcumin and phyllanthin on histone deacetylase 1 (HDAC1), EGFR, Heat Shock Protein 90 Alpha Family Class B Member 1 (HSP90AB1), Hypoxia Inducible Factor 1 Subunit Alpha (HIF1A), and MMP9. The protein structure was obtained from the protein data bank (PDB). Chemical structure of CL and PN was preparation using ChemDraw. The energy-minimized structure of ligand and protein interaction was simulated using AutoDock Vina 1.1.2. The interaction energy between the ligand and the receptor was calculated for the entire binding site and expressed as affinity (kcal/mol). The Pymol program self-generated schematic 2D representations of the interfaces of protein–ligand complexes from standard pdb file input.

### Data analysis

Molecular docking results were validated by determining the RMSD value of conformation bearing the lowest docking score. The validity of the molecular docking method was represented as RMSD value  $<2$ . Cytotoxic potencies against several cell lines were statistically analyzed basing from the  $IC_{50}$  values through linear regression with  $p > 0.05$ . Values were presented as the mean  $\pm$  SD.

## Results

### Cytotoxic effect of single doses of CL and PN in inhibiting MDA-MB-231 cells proliferation

The cytotoxic activities of CL and PN individually and in combination against MDA-MB-231 BC cells were determine used MTT assay in various concentration.

MDA-MB-231 incubates in the presence of 10–200  $\mu\text{g}/\text{ml}$  CL and PN individually for 24 h. In this study, we found that CL and PN in single treatment significantly decreased the cell viability with degree depletion in a dose-dependent manner. A single treatment of CL showed the cytotoxic effect on MDA-MB-231 with  $\text{IC}_{50}$  value of 126  $\mu\text{g}/\text{ml}$  for 24 h (Figure 1a). In addition, the CL also caused morphological changes under inverted microscope observation (Figure 1b). Low concentration of CL has not shown significant morphological changes, but there is clearly a reduction in cell density compared to untreated. Interestingly, in the middle and high concentration of CL induced cell shrinkage, pyknosis, and fragmentation. Cell shrinkage and pyknosis are visible through inverted microscopy during the early process of apoptosis [36]. With cell shrinkage, the size of the cells is smaller, the cytoplasm denser, and the organelles tighter. Pyknosis is the result of the condensation of chromatin and the most characteristic feature of apoptosis [37], [38]. These findings clearly indicated that CL has a potential to reduce cell viability against MDA-MB-231 BC cells may be through apoptosis induction.

The same phenomenon also occurs in the presence of PN. The  $\text{IC}_{50}$  value of PN with respect to MDA-MB-231 was found to be 359  $\mu\text{g}/\text{ml}$  for 24 h (Figure 2a). Under the inverted microscope, only in a high concentration of PN shows that the morphology of detached round cells that floated medium with the bubbled membrane, wrinkled nucleolus, was obviously observed in the MDA-MB-231 cells. Such morphological characteristics are a sign of cell death and have not been seen in the untreated cells (Figure 2b). In this regard, CL had a strong inhibitory effect more than PN. For further exploration we observed the effects of combination CL-PN on MDA-MB-231 cell growth to increase efficacy.

### Synergistic effect of combination CL and PN to inhibit MDA-MB-231 cells proliferation

Cells were treated for 24 h with several concentrations of CL and PN for to investigated the combination effect of CL and PN on the viability of triple negative BC (TNBC) with high population of BCSCs MDA-MB-231 cells. In CL-PN combination, the  $\text{IC}_{50}$  value obtained after single treatment of CL and PN was used to determine their concentration. The concentration used wa calculated as one-half (65  $\mu\text{g}/\text{ml}$ ; 180  $\mu\text{g}/\text{ml}$ ), one-fourths (32.5  $\mu\text{g}/\text{ml}$ ; 90  $\mu\text{g}/\text{ml}$ ), and one-eighth (16.25  $\mu\text{g}/\text{ml}$ ; 45  $\mu\text{g}/\text{ml}$ ) or one-sixteenth (8.125  $\mu\text{g}/\text{ml}$ ; 22.5  $\mu\text{g}/\text{ml}$ ) of CL and PN, respectively. The results indicated that all concentration of either CL (Figure 1a) or PN (Figure 2a) in single treatment inhibited cell growth by <50%.

In contrast to the single treatment of CL and PN, their combination at the four concentrations above showed significantly reduced cell viability

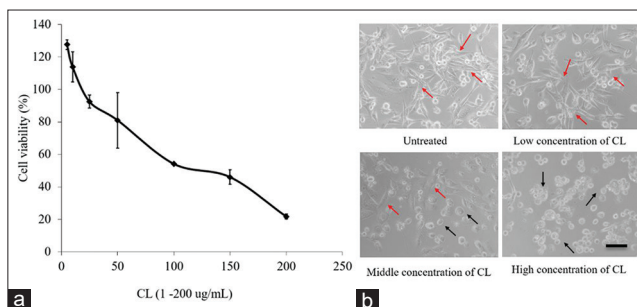


Figure 1: (a) Inhibitory effect of *Curcuma longa* (CL) on MDAMB-231 cells proliferation. MDAMB-231 cells viability was measured after 24 h of CL. Cell viability profile expressed mean  $\pm$  SD of three experiments.  $\text{IC}_{50}$  obtained from a linear regression calculation of log concentration versus cell viability with  $p < 0.05$ . (b) The effect of CL on cell morphology. Visible morphological changes and population of cells in treatment of untreated, low concentration of CL (5  $\mu\text{g}/\text{ml}$ ), middle concentration of CL (100  $\mu\text{g}/\text{ml}$ ), and high concentration of CL (200  $\mu\text{g}/\text{ml}$ ). Red arrows indicated normal living cells, while black arrow indicate the morphological changes of cells. Observations of cell morphology performed using an inverted microscope with a magnification of  $\times 100$ . Scale bar: 100  $\mu\text{m}$

(Figure 3a). At all concentrations the percentage of viable cells between 50.71% and 5.93%. The smallest number of viable cells was observed with maximum combination concentration of CL and PN (65  $\mu\text{g}/\text{ml}$  and 180  $\mu\text{g}/\text{ml}$ ), respectively. In addition, this combination suppressed doses-dependent manner of MDA-MB-231 cells proliferation. The combination index value of CL-PN was calculated using CompuSyn, these data showed synergistic to very strong synergistic effect with CI value between 0.09 and 0.36 (Table 1). Curve of concentration-effect of CL, PN, and its combination (Figure 4a). The combination index plot showed that all of combination of treatment exhibited a synergism effect, caused the isoboles of combination CL-PN were located to the below of the curve, which indicated that CL-PN

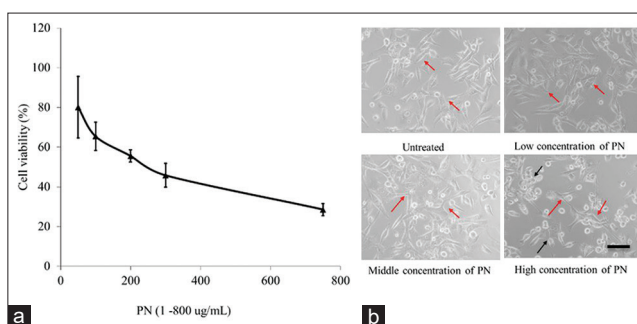


Figure 2: (a) Inhibitory effect of *Phyllanthus niruri* (PN) on MDAMB-231 cells proliferation. MDAMB-231 cells viability was measured after 24 h of PN. Cell viability profile expressed mean  $\pm$  SD of three experiments.  $\text{IC}_{50}$  obtained from a linear regression calculation of log concentration versus cell viability with  $p < 0.05$ . (b) The effect of PN on cell morphology. Visible morphological changes and population of cells in treatment of untreated, low concentration of *Phyllanthus niruri* (PN) (5  $\mu\text{g}/\text{ml}$ ), middle concentration of PN (100  $\mu\text{g}/\text{ml}$ ), and high concentration of PN (200  $\mu\text{g}/\text{ml}$ ). Red arrows indicated normal living cells, while black arrow indicate the morphological changes of cells. Observations of Cell morphology performed using an inverted microscope with a magnification of  $\times 100$ . Scale bar: 100  $\mu\text{m}$

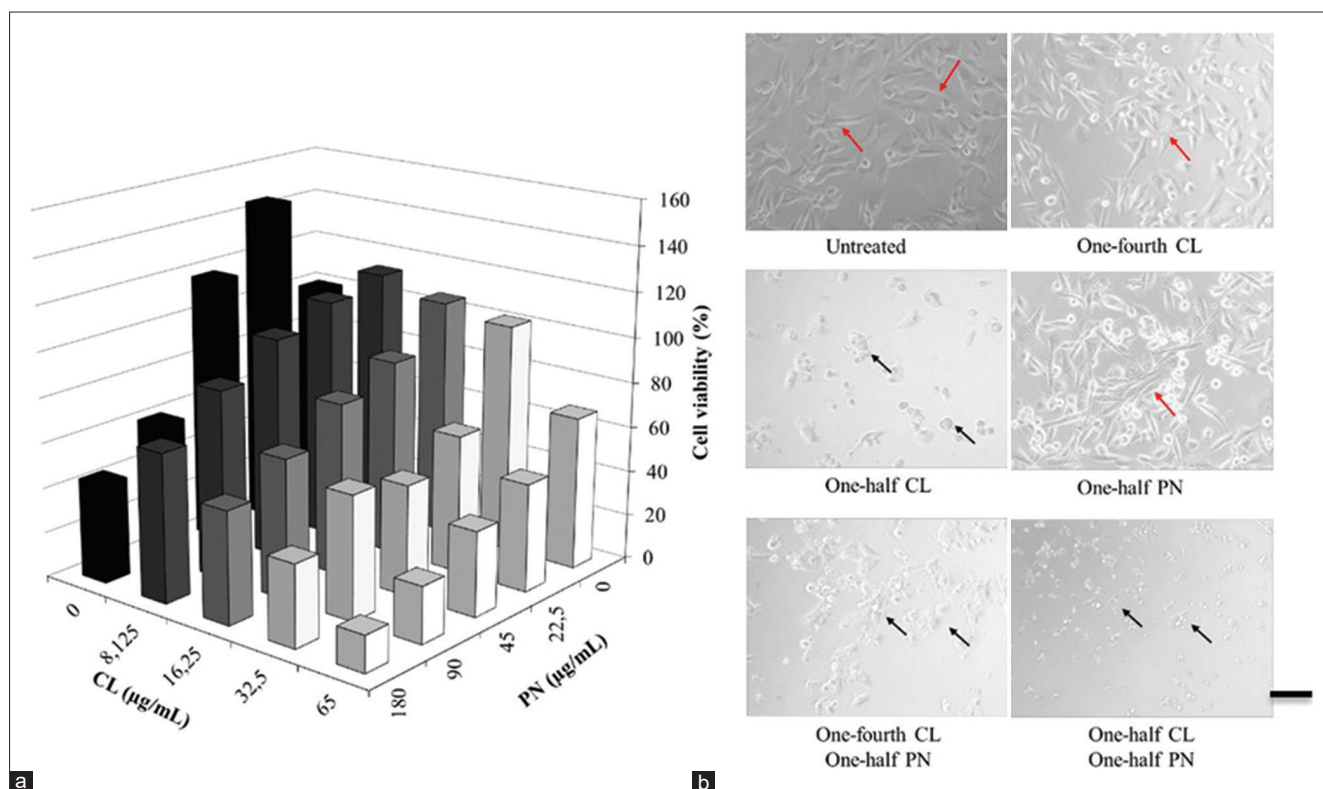


Figure 3: Combination effect of *Curcuma longa*-*Phyllanthus niruri* (CL-PN) on MDA-MB-231 cells. (a) Cells  $5 \times 10^3$  were seeded for 24 h in 96-well plate, then treated with CL, PN in single treatment and its combination for 24 h. Cell viability profile expressed mean  $\pm$  SD of three experiments. (b) The effect of combination CL-PN on cell morphology. Red arrows indicated normal living cells, while black arrow indicate the morphological changes of cells. Observations of Cell morphology performed using an inverted microscope with a magnification of  $\times 100$ . Scale bar: 100  $\mu$ m

has synergism effect (Figure 4b). The viability cells decreased dramatically in the two observed concentration combination (one-half PN-one-fourth CL and one-half PN-one-half CL), marked by unique morphological changes including improvement in the integrity of the membrane, formation of apoptotic bodies, cytoplasmic condensation, and fragmentation of DNA (Figure 3b). It was supported by previous study reported that curcumin a major compound from CL decreased the  $IC_{50}$  of MDA-MB-231 alone and in combination with paclitaxel, cisplatin, or doxorubicin. In addition, curcumin could increase by 15- and 5-fold drug sensitivity of MDA-MB-231 and MCF-7, respectively. BCSCs of MDA-MB-231 proliferate only to the fourth generation under combined treatment of Mitomycin C and curcumin [39]. This is an interesting phenomenon that should be explored further especially a molecular mechanism that involved in this activity, so we carried out the

molecular mechanism exploration using integrative bioinformatics approaches.

**COMPARE analysis reveals the target list of mRNA and the standard agent**

The molecular mechanism of CL and PN in BCSCs was explored using a bioinformatics approach. Curcumin and phyllanthin are the major compounds of CL and PN was used for subject analysis in this approach. The microarray level of mRNA expression analyzed by COMPARE revealed that 147 gene (93 gene with a positive Pearson correlation and 54 gene with a negative Pearson correlation) (Supplementary Table 1) and 200 gene (151 gene with a positive Pearson correlation and 49 gene with a negative Pearson correlation) (Supplementary Table 2) expression in the NCI-60 cell line panel that was affected by treatment with curcumin and phyllanthin, respectively. In addition, MANEAL, HNRNPR, and RPL11 the gene regulated by curcumin and TBL3, PAG1, and ADAM22, the gene regulated by phyllanthin showed the highest Pearson correlation coefficient. A positive correlation coefficient indicates a direct correlation and implies that a higher mRNA expression enhances stemness capability of BCSCs and vice versa. Furthermore, the PubMed search using the keyword “BCSCs” resulted in 1478 gene associated with BCSCs (Supplementary Table 3).

Table 1: Combination index value of CL-PN calculated using CompuSyn

| Concentration | CL (µg/ml) |        |        |        |
|---------------|------------|--------|--------|--------|
|               | 8.125      | 16.25  | 32.5   | 65     |
| PN (µg/ml)    |            |        |        |        |
| 22.5          | 0.29**     | 0.21** | 0.09*  | 0.09*  |
| 45            | 0.11**     | 0.13** | 0.10** | 0.13** |
| 90            | 0.29**     | 0.35#  | 0.18** | 0.24** |
| 180           | 0.22**     | 0.27** | 0.36#  | 0.18** |

\*Combination index value <0.1 indicate very strong synergism, \*\*Combination index value 0.1–0.3 indicate strong synergism, #Combination index value 0.3–0.7 indicated synergism. CL-PN: *Curcuma longa*-*Phyllanthus niruri*.

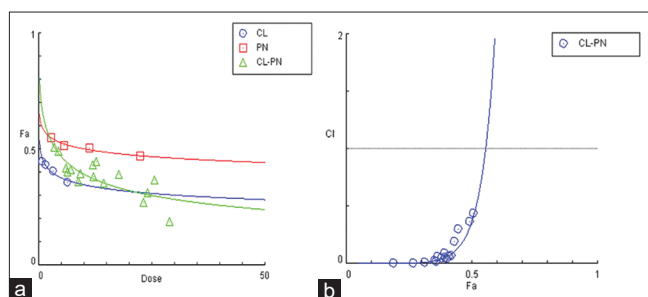


Figure 4: (a) Dose-effect curves of *Curcuma longa*, *Phyllanthus niruri*, and its combination. Dose-effect curves were generated from the CompuSyn calculation, and the value are the mean of three experiment. (b) Combination index plot among 16 combination, all off data point on the synergy side ( $CI < 1$ )

Further, a Venny 2.1 diagram analysis (Figure 5a) of microarray data and PubMed gene list generated 1 gene that was regulated by curcumin, phyllanthin and related to BCSCs, 11 and 9 genes that were related to BCSCs and regulated by curcumin and phyllanthin individually, respectively (Supplementary Table 4). ANXA2 is a gene that was regulated by curcumin and phyllanthin. ANXA2 has the  $-0.52$  Pearson correlation coefficient, it indicated the higher ANXA2 expression, the higher BCSCs formation. We also obtained 10 DTP of curcumin under chemical association network (STITCH) analysis, including CASP3, PTGS2, PPARG, HMOX1, AKT1, TP53, MMP9, STAT3, CCND1, and EGFR (Figure 5b). However, no one of DTP was affected by phyllanthin.

#### GO analysis and KEGG pathway enrichment of potential curcumin and phyllanthin target genes

GO analysis was categorized as cellular component, biological process, and molecular function. 22 gene regulated by curcumin, phyllanthin and related to BCSCs participated in the biological process of protein kinase activity, cadherin binding involved in cell-cell adhesion, cell-cell adherent junction, cell-cell adhesion, and positive regulation of protein phosphorylation. The 22 gene are located in the cell surface, membrane, plasma membrane, nucleus, and nucleoplasm. They also exert a molecular function in ATP binding, DNA binding, and transcriptional factor binding activity (Supplementary Table 4). KEGG pathway enrichment based on FDR of  $< 0.05$  demonstrated various pathways regulated by curcumin and phyllanthin such as cell-cell adhesion, stem cell differentiation, I-kappaB kinase/NF-kappaB signaling, metastatic, and response to oxidative stress. Pathway enrichment analyzed by WebGestalt showed stem cell differentiation regulated by curcumin and phyllanthin (Figure 6). The stem cell differentiation indicated that BCSCs loses of stemness properties and transforms into mature cells [40], [41], [42]. In addition, cell-cell adhesion significantly contributes to cancer metastatic and progression [43]. ANXA2

is involved in adhesion and also regulates the remodeling of ECM [44].

#### PPI analysis of gene regulated by curcumin, phyllanthin and related to BCSCs

The biological role of differential expression genes was examined using STRING data based. PPI networks were constructed of 22 genes consist of 22 nodes, 88 edges, eight average node degree, 0.0697 average local clustering coefficient, and  $< 4.23 \times 10^{-5}$  PPI enrichment p-value (Figure 7a). The top ten genes with the highest score have been identified as HDAC1, EGFR, HSP90AB1, HIF1A, MMP9, PKM, TBK1, TNFAIP3, CHD4, and TCF3 (Figure 7b and c). These proteins correlated with stem cell differentiation and cell proliferation [45], [46].

#### Molecular docking

Induction of stem cell differentiation can be used as a strategic method to overcome stemness of BC cells. And also, this method could better contribute to curing patients [47]. In this study, we conducted a molecular docking simulation to predict the potential inhibitory activity of curcumin and phyllanthin in metastatic and stem cells differentiation pathway. Protein-ligand binding was visualized using Pymol (Figure 8). The ANXA2, HDAC1, EGFR, HSP90AB1, HIF1A, and MMP9 as protein target were picked from the top ten genes with the highest degree score based on their drug target uniqueness. Curcumin showed no binding interaction with HDAC1, it indicated that HDAC1 does not bind nor reacts preferentially with curcumin. However, HDAC1 slightly bind to phyllanthin with docking score value  $-10.4140$ . The lowest docking score of curcumin and phyllanthin was found on HSP90AB1 and the highest docking score of curcumin and phyllanthin was found on MMP9 and HDAC1, respectively (Supplementary Table 5). The lower docking score suggested that highest potential of ligand binding affinity.

#### Discussion

BCSCs as play important role of cancer progression, cancer relapse, metastatic, and drug resistance due to of their ability to self-renew and differentiate into heterogeneous lineages of cancer cells [48]. Cancer relapse and metastatic appear to be the biggest problem for cancer patient management cause the chemotherapy only target the bulk of cancer and are unable to target BCSCs [49]. This is due to the high resistance of BCSCs, which contributes to metastasis and recurrence of cancer.

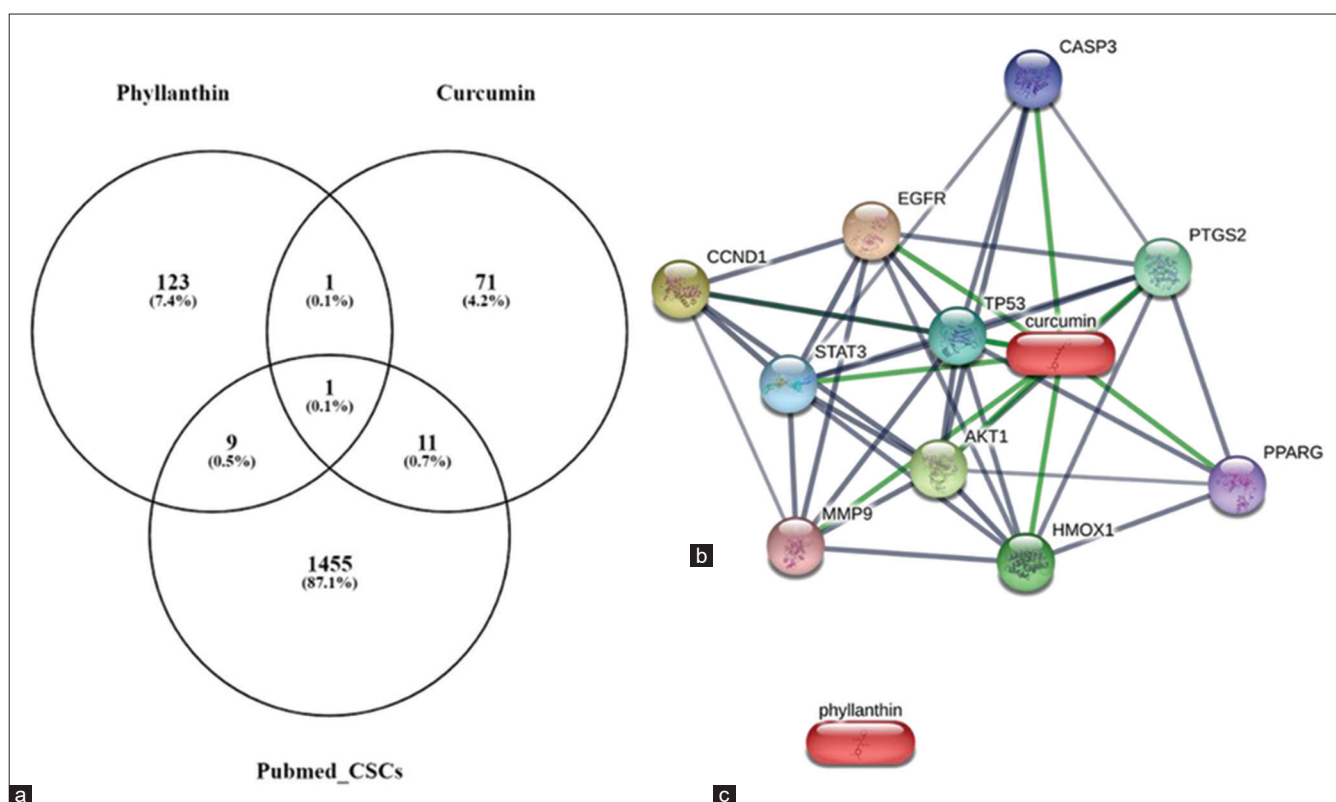


Figure 5: (a) a venn diagram of curcumin and phyllanthin potential targets against BBCSCs. (b) Curcumin interaction and is direct target proteins (DTP). (c) Phyllanthin interaction and its DTP

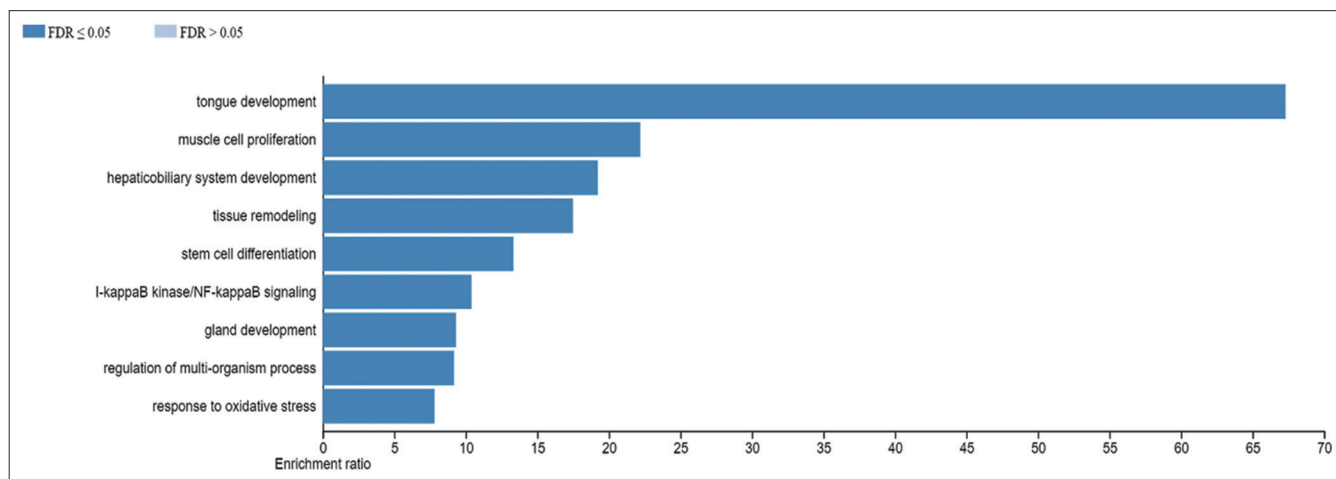


Figure 6: Kyoto encyclopedia of Genes and Genomes pathway enrichment analysis using the Overrepresentation Enrichment Analysis, WebGestalt

Until now chemotherapy remains one of the key therapeutic modalities. However, chemoresistance is limited in effectiveness. Thus, potential therapy to prevent resistance is needed such as using natural chemotherapy. The concepts of natural chemotherapy are intended to enhance efficacy and reduce the side effects of a chemical chemotherapeutic agent [50]. CL and PN are medicinal plants commonly used to treat cancer. CL possess cytotoxic effect on several cancer cells [19], [51], [52]. PN has also been reported to exert many biological effects such as anticancer on various cancer cells [20], [27]. CL and PN have been shown to affect many pathways and factors associated with

tumorigenesis and induced cancer cells death in large targets selectively [53]. However, in recent years the used of herbal medicine is limited as co-chemotherapy. The combination of two herbal medicines is still limited, even though natural compound that is abundant in nature is very potential to be developed further.

The present study resulted in an important finding relevant to the potential of combination natural chemotherapeutic agent, especially in triple negative BC cells (TNBC) with high population of BCSCs. The cytotoxic assay resulted in this study showed that CL and PN are a very strong cytotoxic agent against

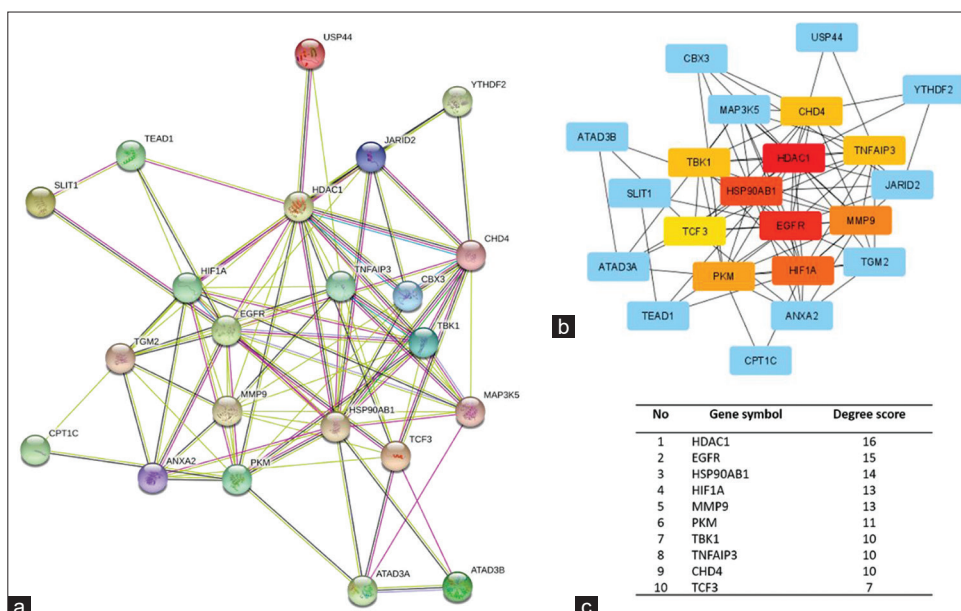


Figure 7: (a) Protein-protein interaction networks of 22 genes analyzed using STRING-DB, (b) Hub genes network analyzed using Cytohubba (red, orange and yellow box indicated the highest degree score of hub genes) and (c) Top ten hub genes based on degree score

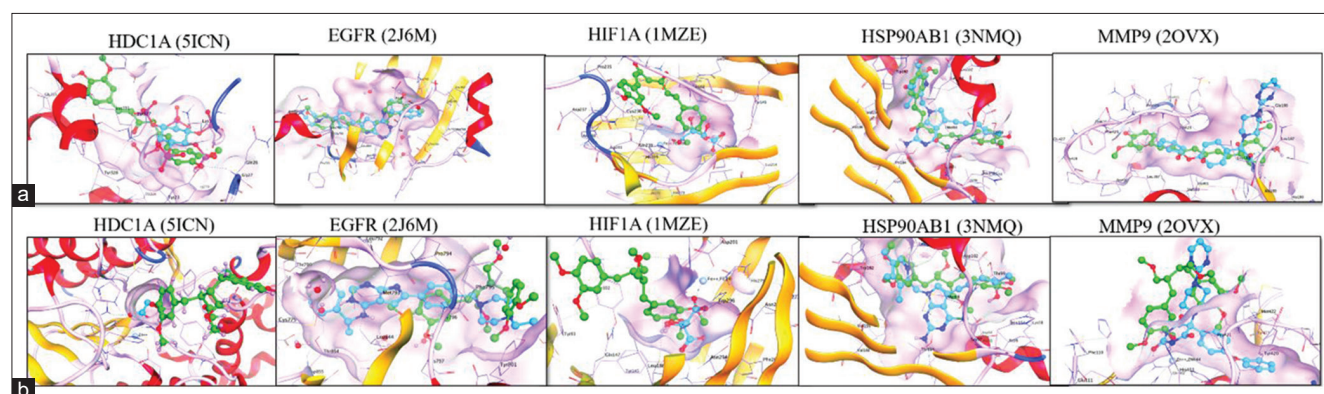


Figure 8: Molecular docking binding conformations (visualized by PyMol software) of top ten hub genes with (a) Curcumin and (b) Phyllanthin

MDA-MB-231 cells. CL and PN normally exhibited *in vitro* anticancer activities through a variety of mechanism in many cancer cells types, including cell cycle arrest and apoptosis induction [54]. CL also leads to an imbalance in the form of the Bcl/Bax associated apoptosis complex, which eventually causes BCSCs to lose their proliferation ability [55]. In addition, the apoptosis induction due to herbal administration through decreased expression of anti-apoptotic protein including surviving [56]. In comparison, PN was reported to be able to inhibited of MMP expression can affect the cell migration and metastasis process [57]. Based on the different mechanism of action of CL and PN, it can be predicted that these activities will be increased in combination therapy. In addition, combination therapies derived from herbal medicine significantly reduce the side effect of drug toxicity and drug resistance. Resistance condition after treatment with chemotherapy prompted the development of new compound called co-chemotherapy, which combined with chemotherapeutic agents. Co-chemotherapy usually consists of one natural compound and chemical

chemotherapy, but in this study, we will combine the two potential herbal compounds without chemotherapy to get a better effect and reduce side effect.

Furthermore, the study was continued to use the combination of CL-PN to investigated combination ability to increase cytotoxic effect. The effect of combination treatment with CL and PN on proliferation depends on their single concentration, suggesting a synergistic effect. Synergy is concluded when the use of drug combinations at different doses results in greater efficacy relative to the amount of the anticancer effects produced by using the individual drugs at the same dose. Combination index values calculated using CompuSyn support this finding, in which the combination index values represent a strong or very strong synergy (combination index 0.09–0.36). Interestingly, the combination of CL-PN showed decreased dramatically of cell viability up to 20.5%. These findings supported by the previous study that curcumin from CL in combination with Mitocymycin C inhibits BCSCs proliferation [39]. Thus, combination of CL and PN could improve the therapeutic effect and



reduce side effect by sensitizing BC cells with high population of BCSCs and may provide a novel approach for cancer therapy. In this study, we also analysis using bioinformatics approaches to explored underlying target and molecular mechanism of the combination CL and PN in BCSCs.

In the bioinformatics study, we used curcumin and phyllanthin as a major compound of CL and PN, to predict tumor responsiveness to a natural product. COMPARE analysis identified 145 and 200 gene regulated by curcumin and phyllanthin, respectively. Curcumin regulated gene of RASAL2, CLIP4, and RFC3 and phyllanthin regulated gene FLJ41649, RND3, and SPATS2L with highest negative Pearson correlation coefficient. A PubMed gene data based found 1478 gene related with BCSCs. Further, a Venn diagram of COMPARE microarray data PubMed gene list produced 22 genes that were regulated by curcumin, phyllanthin and related to BCSCs. ANXA2 is only one gene that was regulated by curcumin and phyllanthin. ANXA2 involve in the cell proliferation, CSC formation and initiated EMT [58]. Indirect downregulated ANXA2 suppress protein levels of stemness-related transcription factors (Nanog, Oct4, and Sox2) through the inhibiting Akt pathway. ANXA2 also promoted the invasion and metastasis [58]. On the other hand, curcumin also exhibit a mechanism of action on several genes individually, MMP9 and EGFR are BCSCs-associated genes and can be directly affected by curcumin. The previous research reported that EGFR pathway play a critical role in regulating BCSCs [45]. Hence, curcumin and phyllanthin are multi-target and have a synergistic effect.

Analysis of KEGG pathway enrichment revealed that stem cell differentiation and NF- $\kappa$ B are regulated by curcumin and phyllanthin. The previous study demonstrated that induced of cancer stem cell differentiation could be better therapy that kills cancer cells. The control of the stem cell differentiation and proliferation signaling pathway play an essential role in killing cancer cells [47]. Moreover, the PPI network reported that 12 genes had a degree score more than 10. HDAC1, EGFR, HSP90AB1, HIF1A, and MMP9 are the five genes with highest degree scores. HIF1A is a master transcriptional regulator of hypoxia condition, which induces EMT and BCSCs niche formation and also chemo and radio resistance of BCSCs. Under hypoxia, HIF1A regulate resistance and metastatic potential to BCSCs. It indicates that HIF1A is critically for survival, self-renewal, and BCSCs growth [59]. Further, the interaction of curcumin and phyllanthin on HIF1A genes in BCSCs may present breakthrough therapy for induced BCSCs differentiation to mature cells. Thus, mature cancer cells could make them easy to kill. In the other hand, activation of EGFR a member of EGFR/ErbB tyrosine kinases family also could increase tumorsphere formation, a characteristic ability of BCSCs [45]. Interestingly, curcumin and phyllanthin

showed high binding affinity with EGFR based on molecular docking simulation. It indicated that the interaction between curcumin and phyllanthin to EGFR could inhibit EGFR activation pathway which impact on inhibited metastasis. The results support the previous findings that treatment with EGFR inhibitor, results in loss of tumorsphere-forming ability [60]. In addition, inhibiting EGFR components of signaling pathway resulting in the reduced survival and higher motility of BCSCs. Curcumin and phyllanthin also showed effect on direct interaction with MMP9. Previously has been reported that downregulation of MMP9 inhibited the cancer invasion. In addition, the previous study also confirms that curcumin induced downregulation of MMP9 through inhibition of I $\kappa$ B- $\alpha$  degradation [61]. Hence, inhibition a cancer BCSCs one of marker stem cells differentiation can be used a strategic method to overcome the stemness. Another mechanism of CL-PN is influence on HSB90AB1 gene. The lowest docking score of curcumin and phyllanthin embedded into HSB90AB1 showed that CL-PN influence on cancer stem cells differentiation pathway and cell migration, invasion and metastasis. A recent study established a crucial role of HSP90AB1 in the stemness development in immune-refractory tumors: The NANOG-driven HSP90A/TCL1A/Akt pathway is responsible for the emergence of CSC-like tumor cells exhibiting an insusceptibility to immune attack, aggressiveness, and multi-modal resistance. Using selective inhibitors of HSP90AB1 such as curcumin and phyllanthin can be conductive EMT in carcinoma of different localization. Hence, inhibition of intracellular HSP90AB1 was reported to inhibit EMT via activation of HIF1A and NF- $\kappa$ B [62].

## Conclusion

In this study, CL and PN suggested strong synergistic effect to inhibited BC cells proliferation. Moreover, CL and PN in combination showed that induced sensitivity on BC cell line with a high population of BCSCs. More important, using a bioinformatics approach curcumin and phyllanthin a major compound of CL and PN, respectively demonstrated that regulated on stem cell differentiation and metastatic pathway in overcoming stemness in BC. Molecular docking study showed the possible target of curcumin and phyllanthin against stem cell differentiation regulator pathway. Overall, combination of CL and PN has potential for the treatment of BCSCs and the finding of this study could be beneficial for research on accelerating and directing the screening of possible targets and identifying the molecular mechanism of curcumin and phyllanthin to resolve stemness of BCSCs.

## Authors' Contributions

AG and DH contributed to the conception of the work. AG, DH, DM, AL, and NDA contributed to the acquisition of the work. DH and NDA contributed to the analysis and interpretation of data. AG and NDA contributed to drafting the work. AG, DNA, and DH contributed to revising the work critically. DM contributed to the revising of the manuscript. AG is responsible for giving the final approval of the manuscript.

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PMid:32268506

### Author Query???

AQ1: We are able to not process this table no 3. please check and advise

AQ2: Kindly provide supplementary Table header

**AQ1** **Supplementary Tables**

**AQ2** **supplementary Table 1 ???**

| Rank | Correlation | Seed vector<br>description for | Target Vector idebt<br>For display | Target vector<br>Display for |
|------|-------------|--------------------------------|------------------------------------|------------------------------|
| 57   | 0.52        | CURCUMIN                       | GC382441                           | CHD4                         |
| 58   | 0.52        | CURCUMIN                       | GC382639                           | KHDRBS1                      |
| 60   | 0.52        | CURCUMIN                       | GC415957                           | U2AF1                        |
| 61   | 0.52        | CURCUMIN                       | GC397232                           | SMARCC1                      |
| 62   | 0.52        | CURCUMIN                       | GC16214                            | CPT1C                        |
| 63   | 0.52        | CURCUMIN                       | GC32693                            | TAF12                        |
| 64   | 0.52        | CURCUMIN                       | GC410576                           | RPF2                         |
| 65   | 0.52        | CURCUMIN                       | GC397403                           | HDAC1                        |
| 66   | 0.52        | CURCUMIN                       | GC257044                           | RBBP4                        |
| 67   | 0.52        | CURCUMIN                       | GC397651                           | KHDRBS1                      |
| 69   | 0.52        | CURCUMIN                       | GC255719                           | TCF3                         |
| 70   | 0.52        | CURCUMIN                       | GC16168                            | PPP1R8                       |
| 72   | 0.51        | CURCUMIN                       | GC56403                            | CHEK2                        |
| 73   | 0.51        | CURCUMIN                       | GC184242                           | RABEPK                       |
| 74   | 0.51        | CURCUMIN                       | GC382464                           | SRRM1                        |
| 76   | 0.51        | CURCUMIN                       | GC282748                           | C22orf34                     |
| 77   | 0.51        | CURCUMIN                       | GC382380                           | TRA2B                        |
| 79   | 0.51        | CURCUMIN                       | GC171597                           | HNRNPR                       |
| 80   | 0.51        | CURCUMIN                       | GC187777                           | ATAD3A                       |
| 82   | 0.51        | CURCUMIN                       | GC384395                           | CCNF                         |
| 83   | 0.51        | CURCUMIN                       | GC384141                           | ZBTB40                       |
| 84   | 0.51        | CURCUMIN                       | GC264147                           | RBBP4                        |
| 85   | 0.51        | CURCUMIN                       | GC393435                           | S100BPB                      |
| 86   | 0.51        | CURCUMIN                       | GC257220                           | ODF2                         |
| 87   | 0.51        | CURCUMIN                       | GC35203                            | NFATC2IP                     |
| 88   | 0.51        | CURCUMIN                       | GC37811                            | SLC25A36                     |
| 90   | 0.51        | CURCUMIN                       | GC85211                            | ATAD3B                       |
| 91   | 0.51        | CURCUMIN                       | GC421500                           | C1orf174                     |
| 92   | 0.51        | CURCUMIN                       | GC396851                           | SRSF4                        |
| 95   | 0.51        | CURCUMIN                       | GC383951                           | PCNT                         |
| 96   | 0.51        | CURCUMIN                       | GC384561                           | ZZEF1                        |
| 97   | 0.5         | CURCUMIN                       | GC386207                           | SLC19A1                      |
| 99   | 0.5         | CURCUMIN                       | GC270113                           | ZNRD1                        |
| 100  | 0.5         | CURCUMIN                       | GC31073                            | BTAF1                        |
| 102  | 0.5         | CURCUMIN                       | GC15330                            | PNRC2                        |
| 105  | 0.5         | CURCUMIN                       | GC171761                           | ATAD3B                       |
| 106  | 0.5         | CURCUMIN                       | GC257194                           | GRK4                         |
| 107  | 0.5         | CURCUMIN                       | GC102206                           | HNRNPA2B1                    |
| 108  | 0.5         | CURCUMIN                       | GC391447                           | CCNL1                        |
| 109  | 0.5         | CURCUMIN                       | GC98228                            | DCP1A                        |
| 110  | 0.5         | CURCUMIN                       | GC397043                           | SDHB                         |
| 112  | 0.5         | CURCUMIN                       | GC382062                           | DDX39B                       |
| 114  | 0.5         | CURCUMIN                       | GC391207                           | PDS1                         |
| 115  | 0.5         | CURCUMIN                       | GC408960                           | RCC2                         |
| 116  | 0.5         | CURCUMIN                       | GC392212                           | MRT04                        |
| 117  | 0.5         | CURCUMIN                       | GC96349                            | SLC19A1                      |
| 1    | -0.63       | CURCUMIN                       | GC265288                           | RASAL2                       |
| 2    | -0.59       | CURCUMIN                       | GC281649                           | RASAL2                       |
| 3    | -0.59       | CURCUMIN                       | GC405831                           | CLIP4                        |
| 4    | -0.58       | CURCUMIN                       | GC409778                           | CLIP4                        |
| 5    | -0.57       | CURCUMIN                       | GC14289                            | RFC3                         |
| 6    | -0.57       | CURCUMIN                       | GC9827                             | TMCO1                        |
| 9    | -0.55       | CURCUMIN                       | GC10417                            | GIGYF2                       |
| 10   | -0.55       | CURCUMIN                       | GC98923                            | RASAL2                       |
| 11   | -0.55       | CURCUMIN                       | GC392962                           | TMEM245                      |
| 12   | -0.54       | CURCUMIN                       | GC92198                            | RASAL2                       |
| 13   | -0.54       | CURCUMIN                       | GC175647                           | RASAL2                       |
| 15   | -0.54       | CURCUMIN                       | GC189569                           | CLIP4                        |
| 16   | -0.54       | CURCUMIN                       | GC160964                           | AHNAK2                       |
| 17   | -0.54       | CURCUMIN                       | GC388269                           | EGFR                         |
| 18   | -0.53       | CURCUMIN                       | GC54614                            | WWC1                         |
| 20   | -0.53       | CURCUMIN                       | GC387675                           | EGFR                         |
| 21   | -0.53       | CURCUMIN                       | GC67614                            | RASAL2                       |
| 22   | -0.53       | CURCUMIN                       | GC395520                           | LOC100288911                 |
| 23   | -0.53       | CURCUMIN                       | GC73287                            | CLIP4                        |
| 24   | -0.53       | CURCUMIN                       | GC283235                           | LOC100288911                 |
| 26   | -0.53       | CURCUMIN                       | GC272846                           | CLIP4                        |
| 27   | -0.53       | CURCUMIN                       | GC269551                           | RASAL2                       |
| 28   | -0.52       | CURCUMIN                       | GC78555                            | C15orf52                     |
| 29   | -0.52       | CURCUMIN                       | GC151105                           | WWC1                         |
| 30   | -0.52       | CURCUMIN                       | GC412092                           | FAM200B                      |
| 31   | -0.52       | CURCUMIN                       | GC249495                           | CRIM1                        |
| 32   | -0.52       | CURCUMIN                       | GC162374                           | RASAL2                       |
| 33   | -0.52       | CURCUMIN                       | GC279709                           | ARHGEF28                     |
| 34   | -0.52       | CURCUMIN                       | GC383037                           | EGFR                         |
| 35   | -0.52       | CURCUMIN                       | GC246094                           | ANXA2                        |
| 39   | -0.51       | CURCUMIN                       | GC85752                            | ANXA2                        |
| 41   | -0.51       | CURCUMIN                       | GC255693                           | ANXA2P2                      |
| 43   | -0.51       | CURCUMIN                       | GC283236                           | LOC100288911                 |
| 44   | -0.51       | CURCUMIN                       | GC15805                            | HIF1A                        |
| 45   | -0.51       | CURCUMIN                       | GC382912                           | EGFR                         |
| 46   | -0.51       | CURCUMIN                       | GC183517                           | RASAL2                       |
| 47   | -0.51       | CURCUMIN                       | GC419198                           | LOC100288911                 |
| 49   | -0.51       | CURCUMIN                       | GC179764                           | RASAL2                       |
| 50   | -0.51       | CURCUMIN                       | GC269692                           | TMEM245                      |
| 52   | -0.51       | CURCUMIN                       | GC271961                           | HIBADH                       |
| 54   | -0.5        | CURCUMIN                       | GC397070                           | HIF1A                        |

(Contd)

**supplementary Table I: (Continued)**

| Rank | Correlation | Seed vector<br>description for | Target Vector idebt<br>For display | Target vector<br>Display for |
|------|-------------|--------------------------------|------------------------------------|------------------------------|
| 55   | -0.5        | CURCUMIN                       | GC274893                           | CRIM1                        |
| 56   | -0.5        | CURCUMIN                       | GC402719                           | AHNAK2                       |
| 57   | -0.5        | CURCUMIN                       | GC384111                           | THBD                         |
| 58   | -0.5        | CURCUMIN                       | GC269658                           | TMEM245                      |
| 59   | -0.5        | CURCUMIN                       | GC267119                           | CLIP4                        |
| 60   | -0.5        | CURCUMIN                       | GC101811                           | null                         |
| 61   | -0.5        | CURCUMIN                       | GC269693                           | TMEM245                      |
| 62   | -0.5        | CURCUMIN                       | GC249354                           | TNFAIP2                      |
| 63   | -0.5        | CURCUMIN                       | GC177839                           | null                         |
| 64   | -0.5        | CURCUMIN                       | GC28815                            | TGM2                         |
| 65   | -0.5        | CURCUMIN                       | GC392961                           | TMEM245                      |
| 66   | -0.5        | CURCUMIN                       | GC269694                           | TMEM245                      |
| 67   | -0.5        | CURCUMIN                       | GC268109                           | ANKRD2                       |
| 1    | 0.72        | CURCUMIN                       | GC16324                            | MANEAL                       |
| 2    | 0.64        | CURCUMIN                       | GC386657                           | HNRNPR                       |
| 3    | 0.63        | CURCUMIN                       | GC397562                           | RPL11                        |
| 5    | 0.62        | CURCUMIN                       | GC386658                           | HNRNPR                       |
| 6    | 0.6         | CURCUMIN                       | GC170494                           | ATAD3B                       |
| 7    | 0.6         | CURCUMIN                       | GC397563                           | RPL11                        |
| 8    | 0.59        | CURCUMIN                       | GC15534                            | PPP1R8                       |
| 9    | 0.59        | CURCUMIN                       | GC385739                           | PPP1R8                       |
| 10   | 0.59        | CURCUMIN                       | GC391502                           | ATAD3A                       |
| 11   | 0.59        | CURCUMIN                       | GC393846                           | ATAD3A                       |
| 13   | 0.57        | CURCUMIN                       | GC388725                           | SRSF10                       |
| 14   | 0.57        | CURCUMIN                       | GC33307                            | RBBP4                        |
| 16   | 0.57        | CURCUMIN                       | GC390415                           | PNRC2                        |
| 17   | 0.57        | CURCUMIN                       | GC392815                           | ATAD3B                       |
| 18   | 0.57        | CURCUMIN                       | GC11583                            | MMP9                         |
| 19   | 0.56        | CURCUMIN                       | GC30216                            | HNRNPR                       |
| 20   | 0.56        | CURCUMIN                       | GC164030                           | ATAD3A                       |
| 22   | 0.55        | CURCUMIN                       | GC265230                           | TMEM39B                      |
| 23   | 0.55        | CURCUMIN                       | GC386665                           | TCP1                         |
| 24   | 0.55        | CURCUMIN                       | GC385792                           | SRSF10                       |
| 25   | 0.55        | CURCUMIN                       | GC393018                           | YTHDF2                       |
| 26   | 0.55        | CURCUMIN                       | GC396451                           | STAG1                        |
| 28   | 0.55        | CURCUMIN                       | GC392055                           | COQ3                         |
| 29   | 0.55        | CURCUMIN                       | GC382442                           | CHD4                         |
| 30   | 0.55        | CURCUMIN                       | GC37945                            | DFFB                         |
| 32   | 0.55        | CURCUMIN                       | GC397652                           | KHDRBS1                      |
| 33   | 0.55        | CURCUMIN                       | GC13127                            | HNRNPR                       |
| 34   | 0.54        | CURCUMIN                       | GC383648                           | MDC1                         |
| 35   | 0.54        | CURCUMIN                       | GC14383                            | SPI1                         |
| 37   | 0.54        | CURCUMIN                       | GC27389                            | SRSF4                        |
| 38   | 0.54        | CURCUMIN                       | GC247512                           | RPL11                        |
| 39   | 0.54        | CURCUMIN                       | GC396479                           | HSP90AB1                     |
| 40   | 0.54        | CURCUMIN                       | GC413387                           | SNHG12                       |
| 41   | 0.54        | CURCUMIN                       | GC48107                            | DCP1A                        |
| 42   | 0.54        | CURCUMIN                       | GC382534                           | SMARCC1                      |
| 43   | 0.54        | CURCUMIN                       | GC384140                           | ZBTB40                       |
| 45   | 0.53        | CURCUMIN                       | GC30500                            | MTHFD2                       |
| 46   | 0.53        | CURCUMIN                       | GC31808                            | SNRNP40                      |
| 47   | 0.53        | CURCUMIN                       | GC413381                           | SNHG12                       |
| 48   | 0.53        | CURCUMIN                       | GC386272                           | SLC19A1                      |
| 49   | 0.53        | CURCUMIN                       | GC421454                           | YARS                         |
| 50   | 0.53        | CURCUMIN                       | GC265256                           | DAZAP1                       |
| 51   | 0.53        | CURCUMIN                       | GC31222                            | AK2                          |
| 52   | 0.53        | CURCUMIN                       | GC394915                           | ATAD3B                       |
| 53   | 0.53        | CURCUMIN                       | GC382463                           | SRRM1                        |
| 55   | 0.53        | CURCUMIN                       | GC383649                           | MDC1                         |
| 56   | 0.53        | CURCUMIN                       | GC255478                           | HNRNPR                       |

## AQ2 supplementary Table 2 ???

| Rank | Correlation | Seed vector<br>description for | Target Vector idebt<br>For display | Target vector<br>Display for |
|------|-------------|--------------------------------|------------------------------------|------------------------------|
| 94   | 0.64        | PHYLLANTHIN                    | GC411175                           | ABHD17B                      |
| 74   | -0.5        | PHYLLANTHIN                    | GC400089                           | CRYGC                        |
| 73   | -0.5        | PHYLLANTHIN                    | GC77299                            | KLHL26                       |
| 76   | -0.5        | PHYLLANTHIN                    | GC71505                            | SETD6                        |
| 59   | -0.51       | PHYLLANTHIN                    | GC57513                            | ACER2                        |
| 63   | -0.51       | PHYLLANTHIN                    | GC13957                            | AVPI1                        |
| 61   | -0.51       | PHYLLANTHIN                    | GC382090                           | CANX                         |
| 70   | -0.51       | PHYLLANTHIN                    | GC89176                            | CDH8                         |
| 62   | -0.51       | PHYLLANTHIN                    | GC96853                            | CLTCL1                       |
| 69   | -0.51       | PHYLLANTHIN                    | GC269143                           | GN3G                         |
| 66   | -0.51       | PHYLLANTHIN                    | GC18210                            | GSTT2B                       |
| 64   | -0.51       | PHYLLANTHIN                    | GC12560                            | HOXB5                        |
| 65   | -0.51       | PHYLLANTHIN                    | GC100193                           | KCNA6                        |
| 60   | -0.51       | PHYLLANTHIN                    | GC63286                            | KIAA1257                     |
| 57   | -0.51       | PHYLLANTHIN                    | GC183485                           | KIF3B                        |
| 71   | -0.51       | PHYLLANTHIN                    | GC253885                           | ZNF205                       |
| 50   | -0.52       | PHYLLANTHIN                    | GC388558                           | ANXA2                        |
| 55   | -0.52       | PHYLLANTHIN                    | GC382841                           | ANXA2                        |
| 56   | -0.52       | PHYLLANTHIN                    | GC387641                           | ANXA2                        |
| 52   | -0.52       | PHYLLANTHIN                    | GC100505                           | CFHR2                        |
| 46   | -0.52       | PHYLLANTHIN                    | GC74837                            | FRG1B                        |
| 53   | -0.52       | PHYLLANTHIN                    | GC10662                            | MAP3K5                       |
| 54   | -0.52       | PHYLLANTHIN                    | GC39938                            | RHO                          |
| 51   | -0.52       | PHYLLANTHIN                    | GC12826                            | SLC25A22                     |
| 44   | -0.53       | PHYLLANTHIN                    | GC54897                            | DOPEY1                       |
| 42   | -0.53       | PHYLLANTHIN                    | GC12470                            | GCA                          |
| 39   | -0.53       | PHYLLANTHIN                    | GC89555                            | HOXC4                        |
| 40   | -0.53       | PHYLLANTHIN                    | GC14484                            | LRRC49                       |
| 41   | -0.53       | PHYLLANTHIN                    | GC17732                            | LYVE1                        |
| 43   | -0.53       | PHYLLANTHIN                    | GC17779                            | SERPINH1                     |
| 34   | -0.54       | PHYLLANTHIN                    | GC42321                            | CPNE8                        |
| 30   | -0.54       | PHYLLANTHIN                    | GC85757                            | GRM5                         |
| 31   | -0.54       | PHYLLANTHIN                    | GC67438                            | KIAA2013                     |
| 37   | -0.54       | PHYLLANTHIN                    | GC9809                             | MYO1E                        |
| 32   | -0.54       | PHYLLANTHIN                    | GC418433                           | NUDT16                       |
| 33   | -0.54       | PHYLLANTHIN                    | GC14218                            | PPP1R14C                     |
| 35   | -0.54       | PHYLLANTHIN                    | GC14031                            | TEAD1                        |
| 24   | -0.55       | PHYLLANTHIN                    | GC16013                            | FAM129B                      |
| 26   | -0.55       | PHYLLANTHIN                    | GC78064                            | KIAA1432                     |
| 28   | -0.55       | PHYLLANTHIN                    | GC397418                           | PKM                          |
| 22   | -0.56       | PHYLLANTHIN                    | GC55618                            | BRAP                         |
| 21   | -0.56       | PHYLLANTHIN                    | GC18216                            | CAPN12                       |
| 23   | -0.56       | PHYLLANTHIN                    | GC16244                            | RALB                         |
| 20   | -0.56       | PHYLLANTHIN                    | GC44018                            | STARD13-AS                   |
| 16   | -0.57       | PHYLLANTHIN                    | GC91520                            | HACE1                        |
| 17   | -0.57       | PHYLLANTHIN                    | GC12073                            | SPECC1                       |
| 15   | -0.58       | PHYLLANTHIN                    | GC11826                            | HAO1                         |
| 13   | -0.58       | PHYLLANTHIN                    | GC16297                            | IGFBP6                       |
| 14   | -0.58       | PHYLLANTHIN                    | GC16157                            | TBK1                         |
| 12   | -0.59       | PHYLLANTHIN                    | GC101167                           | KRT83                        |
| 195  | 0.6         | PHYLLANTHIN                    | GC181232                           | CD1D                         |
| 200  | 0.6         | PHYLLANTHIN                    | GC258803                           | CHRNA3                       |
| 197  | 0.6         | PHYLLANTHIN                    | GC390513                           | EVL                          |
| 194  | 0.6         | PHYLLANTHIN                    | GC387446                           | ITK                          |
| 196  | 0.6         | PHYLLANTHIN                    | GC389990                           | PMS2P1                       |
| 184  | 0.61        | PHYLLANTHIN                    | GC265275                           | ATAD2                        |
| 185  | 0.61        | PHYLLANTHIN                    | GC260950                           | AZ1                          |
| 180  | 0.61        | PHYLLANTHIN                    | GC401477                           | BPTF                         |
| 176  | 0.61        | PHYLLANTHIN                    | GC422381                           | CBX2                         |
| 168  | 0.61        | PHYLLANTHIN                    | GC28493                            | CBX3                         |
| 175  | 0.61        | PHYLLANTHIN                    | GC37934                            | CD1A                         |
| 174  | 0.61        | PHYLLANTHIN                    | GC180872                           | CHD1                         |
| 186  | 0.61        | PHYLLANTHIN                    | GC257084                           | CHRNA3                       |
| 192  | 0.61        | PHYLLANTHIN                    | GC183459                           | CYTH1                        |
| 178  | 0.61        | PHYLLANTHIN                    | GC288491                           | ESCO2                        |
| 173  | 0.61        | PHYLLANTHIN                    | GC273466                           | FYB                          |
| 162  | 0.61        | PHYLLANTHIN                    | GC81244                            | FYB                          |
| 170  | 0.61        | PHYLLANTHIN                    | GC383807                           | JARID2                       |
| 169  | 0.61        | PHYLLANTHIN                    | GC177074                           | JARID2                       |
| 191  | 0.61        | PHYLLANTHIN                    | GC154719                           | LOC728485                    |
| 160  | 0.61        | PHYLLANTHIN                    | GC406275                           | LPAR6                        |
| 165  | 0.61        | PHYLLANTHIN                    | GC26995                            | ME2                          |
| 179  | 0.61        | PHYLLANTHIN                    | GC401644                           | PIKFYVE                      |
| 166  | 0.61        | PHYLLANTHIN                    | GC34575                            | PMS2P1                       |
| 190  | 0.61        | PHYLLANTHIN                    | GC152451                           | RASD1                        |
| 161  | 0.61        | PHYLLANTHIN                    | GC166154                           | SEPT6                        |
| 159  | 0.61        | PHYLLANTHIN                    | GC386647                           | SLIT1                        |
| 187  | 0.61        | PHYLLANTHIN                    | GC403069                           | SMA4                         |
| 181  | 0.61        | PHYLLANTHIN                    | GC398843                           | STAU2                        |
| 182  | 0.61        | PHYLLANTHIN                    | GC275611                           | TBCD                         |
| 189  | 0.61        | PHYLLANTHIN                    | GC383847                           | TFDP2                        |
| 183  | 0.61        | PHYLLANTHIN                    | GC37747                            | TFDP2                        |
| 164  | 0.61        | PHYLLANTHIN                    | GC410258                           | TFDP2                        |
| 157  | 0.61        | PHYLLANTHIN                    | GC167809                           | TNRC6C                       |
| 163  | 0.61        | PHYLLANTHIN                    | GC189678                           | TREML2                       |
| 158  | 0.61        | PHYLLANTHIN                    | GC166321                           | ZNF273                       |
| 11   | -0.61       | PHYLLANTHIN                    | GC51594                            | CAAP1                        |
| 152  | 0.62        | PHYLLANTHIN                    | GC188756                           | ADAM22                       |
| 143  | 0.62        | PHYLLANTHIN                    | GC386719                           | ADAM22                       |
| 154  | 0.62        | PHYLLANTHIN                    | GC274494                           | ANXA2R                       |

(Contd)

## supplementary Table 2: Continued

| Rank | Correlation | Seed vector<br>description for | Target Vector idebt<br>For display | Target vector<br>Display for |
|------|-------------|--------------------------------|------------------------------------|------------------------------|
| 140  | 0.62        | PHYLLANTHIN                    | GC273918                           | ATG4D                        |
| 133  | 0.62        | PHYLLANTHIN                    | GC164346                           | DKFZP58611420                |
| 135  | 0.62        | PHYLLANTHIN                    | GC191274                           | DTX2P1-UPK3BP1-PMS2P11       |
| 151  | 0.62        | PHYLLANTHIN                    | GC177885                           | ITK                          |
| 131  | 0.62        | PHYLLANTHIN                    | GC420450                           | LRRC70                       |
| 149  | 0.62        | PHYLLANTHIN                    | GC67065                            | MOB1B                        |
| 138  | 0.62        | PHYLLANTHIN                    | GC261095                           | MTMR4                        |
| 145  | 0.62        | PHYLLANTHIN                    | GC177974                           | PMS2P1                       |
| 155  | 0.62        | PHYLLANTHIN                    | GC32017                            | PPP1R16B                     |
| 147  | 0.62        | PHYLLANTHIN                    | GC410867                           | RBM33                        |
| 132  | 0.62        | PHYLLANTHIN                    | GC259281                           | SEPT6                        |
| 146  | 0.62        | PHYLLANTHIN                    | GC100902                           | SMA5                         |
| 134  | 0.62        | PHYLLANTHIN                    | GC383467                           | TNFAIP3                      |
| 150  | 0.62        | PHYLLANTHIN                    | GC30781                            | YPEL1                        |
| 116  | 0.63        | PHYLLANTHIN                    | GC83241                            | CCDC69                       |
| 128  | 0.63        | PHYLLANTHIN                    | GC81436                            | GNA13                        |
| 122  | 0.63        | PHYLLANTHIN                    | GC250372                           | JARID2                       |
| 123  | 0.63        | PHYLLANTHIN                    | GC54687                            | KIAA0922                     |
| 118  | 0.63        | PHYLLANTHIN                    | GC30354                            | KIAA0922                     |
| 121  | 0.63        | PHYLLANTHIN                    | GC415872                           | MED12L                       |
| 129  | 0.63        | PHYLLANTHIN                    | GC409739                           | PAG1                         |
| 124  | 0.63        | PHYLLANTHIN                    | GC77144                            | PAG1                         |
| 125  | 0.63        | PHYLLANTHIN                    | GC402221                           | PAXIP1                       |
| 120  | 0.63        | PHYLLANTHIN                    | GC253139                           | RAD51C                       |
| 119  | 0.63        | PHYLLANTHIN                    | GC65039                            | ST8SIA4                      |
| 114  | 0.63        | PHYLLANTHIN                    | GC409353                           | USP44                        |
| 126  | 0.63        | PHYLLANTHIN                    | GC387880                           | YME1L1                       |
| 113  | 0.63        | PHYLLANTHIN                    | GC36980                            | ZNF43                        |
| 90   | 0.64        | PHYLLANTHIN                    | GC385040                           | ADAM22                       |
| 83   | 0.64        | PHYLLANTHIN                    | GC426010                           | ADAM22                       |
| 89   | 0.64        | PHYLLANTHIN                    | GC392913                           | CECR6                        |
| 110  | 0.64        | PHYLLANTHIN                    | GC383438                           | CYTH1                        |
| 105  | 0.64        | PHYLLANTHIN                    | GC160896                           | GNA13                        |
| 111  | 0.64        | PHYLLANTHIN                    | GC183619                           | JARID2                       |
| 107  | 0.64        | PHYLLANTHIN                    | GC413619                           | LOC100505555                 |
| 82   | 0.64        | PHYLLANTHIN                    | GC259441                           | MTMR4                        |
| 84   | 0.64        | PHYLLANTHIN                    | GC388787                           | NEU3                         |
| 109  | 0.64        | PHYLLANTHIN                    | GC176422                           | PAG1                         |
| 96   | 0.64        | PHYLLANTHIN                    | GC272411                           | PAG1                         |
| 98   | 0.64        | PHYLLANTHIN                    | GC255705                           | PECAM1                       |
| 104  | 0.64        | PHYLLANTHIN                    | GC264365                           | PLEKHO1                      |
| 92   | 0.64        | PHYLLANTHIN                    | GC252616                           | PPM1E                        |
| 86   | 0.64        | PHYLLANTHIN                    | GC168689                           | SEPT6                        |
| 99   | 0.64        | PHYLLANTHIN                    | GC290826                           | SLC8A1                       |
| 79   | 0.64        | PHYLLANTHIN                    | GC388591                           | SMA5                         |
| 91   | 0.64        | PHYLLANTHIN                    | GC186043                           | STAU2                        |
| 85   | 0.64        | PHYLLANTHIN                    | GC249191                           | TNFAIP3                      |
| 80   | 0.64        | PHYLLANTHIN                    | GC81700                            | TRAT1                        |
| 106  | 0.64        | PHYLLANTHIN                    | GC403774                           | YPEL1                        |
| 101  | 0.64        | PHYLLANTHIN                    | GC185652                           | YPEL1                        |
| 8    | -0.64       | PHYLLANTHIN                    | GC16525                            | SKP1                         |
| 7    | -0.64       | PHYLLANTHIN                    | GC16842                            | SPATS2L                      |
| 70   | 0.65        | PHYLLANTHIN                    | GC406751                           | ABHD17B                      |
| 69   | 0.65        | PHYLLANTHIN                    | GC254784                           | ADAM22                       |
| 68   | 0.65        | PHYLLANTHIN                    | GC395492                           | BHLHE23                      |
| 63   | 0.65        | PHYLLANTHIN                    | GC388190                           | CHRNA3                       |
| 67   | 0.65        | PHYLLANTHIN                    | GC186950                           | EVL                          |
| 73   | 0.65        | PHYLLANTHIN                    | GC383473                           | KIAA0195                     |
| 66   | 0.65        | PHYLLANTHIN                    | GC37712                            | MGA                          |
| 72   | 0.65        | PHYLLANTHIN                    | GC17785                            | NT5C3B                       |
| 77   | 0.65        | PHYLLANTHIN                    | GC273818                           | PAG1                         |
| 76   | 0.65        | PHYLLANTHIN                    | GC173253                           | PHKA1                        |
| 74   | 0.65        | PHYLLANTHIN                    | GC388613                           | SLC7A1                       |
| 78   | 0.65        | PHYLLANTHIN                    | GC271015                           | USP44                        |
| 64   | 0.65        | PHYLLANTHIN                    | GC39325                            | ZNF85                        |
| 5    | -0.65       | PHYLLANTHIN                    | GC18789                            | RND3                         |
| 56   | 0.66        | PHYLLANTHIN                    | GC402418                           | ADAM22                       |
| 57   | 0.66        | PHYLLANTHIN                    | GC251191                           | ADRBK2                       |
| 51   | 0.66        | PHYLLANTHIN                    | GC391646                           | C17orf80                     |
| 46   | 0.66        | PHYLLANTHIN                    | GC90396                            | CYTH1                        |
| 58   | 0.66        | PHYLLANTHIN                    | GC148113                           | PMS2L2                       |
| 49   | 0.66        | PHYLLANTHIN                    | GC33254                            | PRKCH                        |
| 50   | 0.66        | PHYLLANTHIN                    | GC89979                            | PRKCH                        |
| 52   | 0.66        | PHYLLANTHIN                    | GC31918                            | RAD51C                       |
| 59   | 0.66        | PHYLLANTHIN                    | GC47197                            | TFDP2                        |
| 48   | 0.66        | PHYLLANTHIN                    | GC249190                           | TNFAIP3                      |
| 60   | 0.66        | PHYLLANTHIN                    | GC407968                           | TNRC6C                       |
| 44   | 0.67        | PHYLLANTHIN                    | GC411027                           | ANXA2R                       |
| 41   | 0.67        | PHYLLANTHIN                    | GC189655                           | CCDC102B                     |
| 42   | 0.67        | PHYLLANTHIN                    | GC249516                           | CYTH1                        |
| 38   | 0.67        | PHYLLANTHIN                    | GC409806                           | PAG1                         |
| 39   | 0.67        | PHYLLANTHIN                    | GC260186                           | PAXIP1                       |
| 36   | 0.68        | PHYLLANTHIN                    | GC37018                            | CHRNA3                       |
| 35   | 0.68        | PHYLLANTHIN                    | GC63257                            | EVL                          |
| 31   | 0.68        | PHYLLANTHIN                    | GC409544                           | GNA13                        |
| 37   | 0.68        | PHYLLANTHIN                    | GC161814                           | PAG1                         |
| 34   | 0.68        | PHYLLANTHIN                    | GC73834                            | PAG1                         |
| 33   | 0.68        | PHYLLANTHIN                    | GC35292                            | PMS2P1                       |
| 28   | 0.68        | PHYLLANTHIN                    | GC85849                            | PMS2P1                       |
| 30   | 0.68        | PHYLLANTHIN                    | GC184532                           | PRKCH                        |

(Contd)

supplementary Table 2: *Continued*

| Rank | Correlation | Seed vector<br>description for | Target Vector idebt<br>For display | Target vector<br>Display for |
|------|-------------|--------------------------------|------------------------------------|------------------------------|
| 32   | 0.68        | PHYLLANTHIN                    | GC386035                           | YME1L1                       |
| 18   | 0.69        | PHYLLANTHIN                    | GC17987                            | CD1C                         |
| 21   | 0.69        | PHYLLANTHIN                    | GC16214                            | CPT1C                        |
| 19   | 0.69        | PHYLLANTHIN                    | GC383806                           | JARID2                       |
| 23   | 0.69        | PHYLLANTHIN                    | GC86218                            | KIAA0195                     |
| 25   | 0.69        | PHYLLANTHIN                    | GC164007                           | MTMR4                        |
| 27   | 0.69        | PHYLLANTHIN                    | GC426209                           | SLC8A1                       |
| 2    | -0.69       | PHYLLANTHIN                    | GC10700                            | FLJ41649                     |
| 14   | 0.7         | PHYLLANTHIN                    | GC271745                           | GNA13                        |
| 17   | 0.7         | PHYLLANTHIN                    | GC163365                           | LINC00340                    |
| 16   | 0.7         | PHYLLANTHIN                    | GC387664                           | YME1L1                       |
| 13   | 0.71        | PHYLLANTHIN                    | GC399220                           | PRKCH                        |
| 10   | 0.72        | PHYLLANTHIN                    | GC290660                           | LOC100130476                 |
| 8    | 0.73        | PHYLLANTHIN                    | GC187551                           | C17orf80                     |
| 7    | 0.73        | PHYLLANTHIN                    | GC172216                           | ZNF577                       |
| 5    | 0.74        | PHYLLANTHIN                    | GC186260                           | KIAA0195                     |
| 6    | 0.74        | PHYLLANTHIN                    | GC151093                           | KIAA0825                     |
| 4    | 0.75        | PHYLLANTHIN                    | GC16782                            | TBL3                         |
| 2    | 0.76        | PHYLLANTHIN                    | GC254783                           | ADAM22                       |
| 3    | 0.76        | PHYLLANTHIN                    | GC411749                           | PAG1                         |

Supplementary Table 4: A number of 22 mRNA that were related by curcumin and phyllanthin and were related to BBCSCs

| S. No | Pearson correlation coefficient | Target vector ID | Gene Symbol | Gene name   |
|-------|---------------------------------|------------------|-------------|---|
| 1     | 0.69                            | GC383806         | JARID2      | Jumonji And AT-Rich Interaction Domain Containing 2 |
| 2     | 0.65                            | GC271015         | USP44       | Ubiquitin Carboxyl-terminal hydrolase 44            |
| 3     | 0.64                            | GC249191         | TNFAIP3     | TNF alpha induced protein 3                         |
| 4     | 0.61                            | GC28493          | CBX3        | Chromobox 3   |
| 5     | 0.61                            | GC386647         | SLIT1       | Slit Guidance Ligand 1                              |
| 6     | 0.6                             | GC170494         | ATAD3B      | ATPase Family AAA Domain Containing 3B              |
| 7     | 0.59                            | GC393846         | ATAD3A      | ATPase Family AAA Domain Containing 3A              |
| 8     | 0.57                            | GC11583          | MMP9        | Matrix Metalloproteinase 9                          |
| 9     | 0.55                            | GC382442         | CHD4        | Chromodomain Helicase DNA Binding Protein 4         |
| 10    | 0.55                            | GC393018         | YTHDF2      | YTH N6-Methyladenosine RNA Binding Protein 2        |
| 11    | 0.54                            | GC396479         | HSP90AB1    | Heat Shock Protein 90 Alpha Family Class B Member 1 |
| 12    | 0.52                            | GC397403         | HDAC1       | Histone Deacetylase 1                               |
| 13    | 0.52                            | GC255719         | TCF3        | Transcription Factor 3                              |
| 14    | 0.52                            | GC16214          | CPT1C       | Carnitine Palmitoyltransferase 1C                   |
| 15    | 0.51                            | GC15805          | HIF1A       | Hypoxia Inducible Factor 1 Subunit Alpha            |
| 16    | -0.5                            | GC28815          | TGM2        | Transglutaminase 2                                  |
| 17    | -0.52                           | GC246094         | ANXA2       | Annexin A2  |
| 18    | -0.52                           | GC10662          | MAP3K5      | Mitogen-Activated Protein Kinase Kinase 5           |
| 19    | -0.52                           | GC387675         | EGFR        | Epidermal Growth Factor Receptor                    |
| 20    | -0.54                           | GC14031          | TEAD1       | TEA Domain Transcription Factor 1                   |
| 21    | -0.55                           | GC397418         | PKM         | Pyruvate Kinase M1/2                                |
| 22    | -0.58                           | GC16157          | TBK1        | TANK Binding Kinase 1                               |

Supplementary Table 5: Gene ontology of the 22 potential therapeutic target genes

| Term  | p value               | Genes   |
|---|-----------------------|---|
| Biological process  |                       |   |
| GO: 0000122~negative regulation of transcription from RNA polymerase II promoter          | 0.05878410987680528   | HDAC1, JARID2, TCF3, CHD4                                     |
| GO: 0006351~transcription, DNA-templated  | 0.08869262932347735   | HIF1A, HDAC1, JARID2, CBX3, TCF3, CHD4                        |
| GO: 0045892~negative regulation of transcription, DNA-templated                           | 0.12784550606541756   | HDAC1, JARID2, CBX3   |
| GO: 0045893~positive regulation of transcription, DNA-templated                           | 0.1345965033431493    | HIF1A, HDAC1, TCF3  |
| GO: 0006355~regulation of transcription, DNA-templated                                    | 0.2893336322416595    | HIF1A, HDAC1, TCF3, CHD4                                      |
| GO: 0004672~protein kinase activity   | 0.07263251481178587   | EGFR, MAP3K5, TBK1  |
| GO: 0098641~cadherin binding involved in cell-cell adhesion                               | 0.00530635145361415   | HSP90AB1, PKM, EGFR, ANXA2                                    |
| GO: 0005913~cell-cell adherens junction   | 0.0057898661163517506 | HSP90AB1, PKM, EGFR, ANXA2                                    |
| GO: 0016323~basolateral plasma membrane   | 0.01801155022396305   | HSP90AB1, EGFR, ANXA2   |
| GO: 0098609~cell-cell adhesion  | 0.04454764908303356   | HSP90AB1, PKM, ANXA2  |
| GO: 0044822~poly[A] RNA binding   | 0.16227798926506184   | HSP90AB1, PKM, YTHDF2, ANXA2                                  |
| GO: 0001934~positive regulation of protein phosphorylation                                | 0.010848600290442318  | EGFR, MMP9, ANXA2   |
| GO: 0005654~nucleoplasm   | 0.08871697106273643   | HSP90AB1, HIF1A, HDAC1, JARID2, TEAD1, TCF3, CHD4             |
| GO: 0005654~nucleoplasm   | 0.08871697106273643   | JARID2, TEAD1, TCF3, CHD4                                     |
| GO: 0000790~nuclear chromatin   | 0.0205301891902419    | HDAC1, TCF3, CHD4   |
| GO: 0043234~protein complex   | 0.08074688825784038   | HDAC1, TCF3, CHD4   |
| GO: 0016020~membrane  | 0.47395510617554676   | HSP90AB1, EGFR, CHD4, ANXA2                                   |
| GO: 0005886~plasma membrane   | 0.8850254366828423    | PKM, EGFR, TGM2, ANXA2  |
| GO: 0005615~extracellular space   | 0.1996517577718779    | EGFR, MMP9, SLIT1, ANXA2                                      |
| GO: 0016021~integral component of membrane  | 0.9915053828035031    | CPT1C, EGFR, ATAD3A   |
| GO: 0009986~cell surface  | 0.1280244665061579    | HSP90AB1, EGFR, ANXA2   |
| GO: 0008270~zinc ion binding  | 0.17459658423852453   | MMP9, TNFAIP3, CHD4, USP44                                    |
| GO: 0003677~DNA binding   | 0.3465861347844654    | TEAD1, TNFAIP3, TCF3, CHD4                                    |
| GO: 0003700~transcription factor activity, sequence-specific DNA binding                  | 0.11416783095336286   | HIF1A, HDAC1, TEAD1, TCF3                                     |
| GO: 0008134~transcription factor binding  | 0.0480050925831412    | HIF1A, HDAC1, TCF3  |
| GO: 0005524~ATP binding   | 2.63E-04              | HSP90AB1, PKM, EGFR, MAP3K5, ATAD3A, ATAD3B, TBK1, TGM2, CHD4 |
| GO: 0042826~histone deacetylase binding   | 0.007044468809860209  | HSP90AB1, HIF1A, HDAC1  |
| GO: 0000978~RNA polymerase II core promoter proximal region sequence-specific DNA binding | 0.009259543919357753  | HDAC1, TEAD1, TCF3, CHD4                                      |