

Full Length Research Paper

Evaluation of hepatoprotective, antioxidant and cytotoxic properties of aqueous extract of turmeric rhizome (Turmesac®)

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In the present study, the hepatoprotective, antioxidant and cytotoxic activities of aqueous extract of turmeric rhizome (Turmesac®) were evaluated. In brief, Turmesac® was extracted from turmeric rhizome. 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay was used to evaluate the hepatoprotective and cytotoxic activities of the Turmesac® against the human liver (HUH-7) and breast cancer (MCF-7) cell lines, respectively. The extract was further tested for antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) which was compared with standard butylated hydroxytoluene (BHT). Turmesac® showed significant cytotoxic potential against the MCF-7 cell lines with IC50 values of 163.19 µg/ml and show no cytotoxic potential against the HUH-7 cell lines. Turmesac® was used to stimulate hydrogen peroxide (H₂O₂-200 µM); a dose - dependent suppression of the toxic nature of H₂O₂ by the extract was observed. This confirmed the hepatoprotective potential of Turmesac®. The significant antioxidant activity of Turmesac® and standard BHT was observed at IC50 235.04 and 82.65 µg/mL, respectively. Further investigations will be carried out to evaluate the in vivo hepatoprotective activity of Turmesac®.

Key words: Turmesac®, antioxidant, hepatoprotective, cell viability.

INTRODUCTION

Turmesac (*Curcuma longa* Linn) is a member of the Zingiberaceae family and is cultivated in tropical and subtropical regions around the world. It originates from India, Southeast Asia and Indonesia (Paramasivam et al., 2009). Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies.

Owing to its brilliant yellow color, turmeric is also known as Indian saffron. In the last 25 years, the importance of turmeric has been brought to lime light, as indicated by the over 3000 publications. According to the ayurvedic pharmacopoeia of India, essential oil from the rhizome of *curcuma longa* was used as a carminative, stomachic and tonic (The Ayurvedic Pharmacopoeia of India, 2001). Rhizomes are used as expectorant, cosmeceuticals,

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antiseptic, anthelmintic, blood purifier, and insecticide, spasmolytic and for the treatment of leprosy, hypertensive, cholera, syphilis, spleen disorders rheumatism, bronchitis, cough and cold (Kapoor, 1990). Recent studies have reported that curcumin effectively inhibits liver cirrhosis through its action on many pathways; for example, it inhibits the NF- κ B pathway and reduces oxidative stress (Cai et al., 2017). Curcumin has antioxidant, anti-inflammatory, and anticarcinogenic pharmacological effects (Qiu et al., 2017). It acts by either interacting with molecular targets directly or altering gene expression and signaling pathways. Thus, curcumin has potential as a therapy for liver diseases. Curcumin, a polyphenolic pigment extracted from turmeric is a typical example because of its low toxicity and anticancer potency. The application of curcumin as a complementary therapy for ovarian cancer appears promising, because it induces apoptosis sensitivity to cisplatin in ovarian carcinoma without decreasing quality of life (Zhang et al., 2012; Sadzuka et al., 2012). Moreover, the enhancement of adaptive immunity was involved in curcuma-medicated tumor growth retardation (Luo et al., 2011; Bhattacharyya et al., 2010). In recent years, researches have confirmed that polysaccharides from natural products possess wide- ranging beneficial therapeutic effects and health-promoting properties. Recently, the polysaccharide is reported as a kind of effective free radical scavenger and antioxidant, playing a critical role in protecting against oxidation damage in living organisms. On the other hand, many diseases, such as asthma, chronic obstructive pulmonary disease, inflammation, diabetes, myocardial infarction, and cardiovascular diseases, are reported to be associated with oxidative stress (Scheibmeir et al., 2005; Olmez and Ozyurt, 2012; Sugamura and Keane, 2011). In this study, aqueous extract of turmeric rhizome (Turmesac®) for hepatoprotective, antioxidant and cytotoxic activities were evaluated.

MATERIALS AND METHODS

Collection of plant material

Turmeric rhizome (Salem variety) were collected from Salem, November 2018, Tamilnadu, India and identified by K. Ramesh taxonomist, R &D division, Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India. The turmeric rhizome was dried at room temperature and powdered.

Preparation of the extract

The turmeric powder (250 g) was extracted with distilled water 100°C for 6 h. The extract was filtered, concentrated to remove the water under spray drying and the resulting powder was assayed for various biological activities.

Chemicals and reagents

Human hepatocellular adenocarcinoma (HUH-7) and human breast

adenocarcinoma (MCF-7) cell line were purchased from NCCS, pune, India. Butylated hydroxyl toluene, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 5,5-dithiobis(2-nitrobenzoic acid), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Reagent (Cat No: M6494, Invitrogen), DMSO (Cat No: D4540, Sigma), DMEM-High Glucose, Fetal Bovine Serum (FBS) and Antibiotic-Antimycotic (100x) were procured from Himedia, India. Analytical reagents and chemicals were purchased from Merck, India.

Cell lines and culture medium

The MCF-7 and HUH-7 stock cells were cultured and maintained using 89% DMEM-High glucose supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic (100x) and incubated at 37°C in a humidified atmosphere of 5% CO₂ until the cells reached 70 to 80% confluency. The cells were dissociated with cell 0.25% Trypsin-EDTA.

MTT cytotoxicity assay

The MTT assay determined by the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product (Alley et al., 1986). The cells are then solubilised with an organic solvent (e.g. isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The viability of the cells was checked using Hemacytometer prior to MTT Assay. 200 μ L of cell suspension was seeded in a 96-well plate at the required cell density (20,000 cells per well in media), without the test agent and allowed to grow for about 24 h.

After 24 h, Turmesac® extract (25, 50, 100, 200 and 400 μ g/mL) in media was added to treat the cells and incubated for another 24 h at 37°C in 5% CO₂ atmosphere. Camptothecin with a concentration of 25 μ M was used as a positive control. After the incubation period, the morphology of the cells was observed using inverted biological microscope and images of the cells was captured at 10x magnification. Thereafter, the spent media was removed and 100 μ L of MTT reagent (0.5 mg/mL) was added to each well and incubated for 3 h at 37°C. Thereafter, the formed formazone crystals were dissolved with 100 μ L of DMSO and the absorbance readings were taken by ELISA Reader (ELX 800, Biotek) at 570 nm.

The viability of the cells was determined by the following formula:

$$\% \text{ of viability} = (\text{Mean OD of test compound treated Cells} / \text{Mean OD of untreated cells}) \times 100$$

Hepatoprotective activity

Briefly, 200 μ L of cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent and the cells were allowed to grow for about 24 h. 200 μ M of H₂O₂ was added to all the wells except the untreated wells and incubate for 4 h to stimulate stress to cells and appropriate concentrations of test compound (25, 50, 100, 200 and 400 μ g/mL) was added and incubated for 24 h at 37°C in a 5% CO₂ atmosphere. After the incubation period, 100 μ L of MTT reagent (5 mg/mL) was added and incubated for 3 h at 37°C. After incubation period, the formed formazan crystals were dissolved with 100 μ L of DMSO and the absorbance readings were taken by a spectrophotometer or an

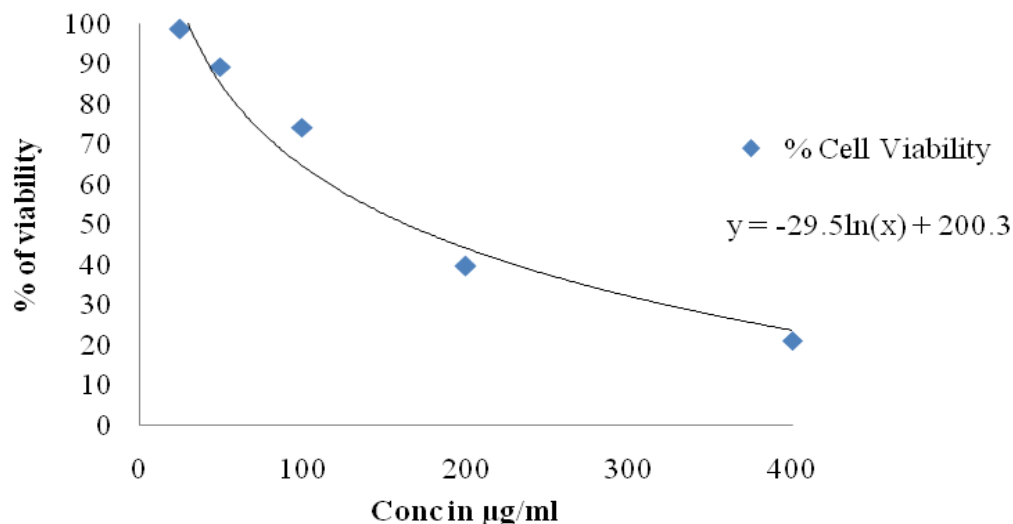


Figure 1. Scatter graph showing the % of cell viability of MCF7 cell line against the Turmesac® and the inhibitory concentration (IC_{50} value) observed is 163.19 $\mu\text{g/ml}$.

ELISA reader at 570 nm (Torres- González et al., 2016). The viability of the cells was determined by the following formula:

$$\% \text{ of viability} = (\text{OD of test compound} / \text{OD of untreated cells}) \times 100$$

Determination of antioxidant activity

The free radical scavenging activity of Turmesac® was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method of Blois (1958). A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (50, 100, 150, 200 and 250 $\mu\text{g/mL}$). After 30 min, absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as the reference material. Percentage DPPH radical scavenging activity was calculated by the following equation. The radical scavenging activity of DPPH was calculated using the following equation:

$$\% \text{ of DPPH radical scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

Ultraviolet detection of turmeric polysaccharides

Preparation of blank solution

1 ml of distilled water was added to 1 ml of 5% phenol followed by 5 ml of concentrated H_2SO_4 .

Preparation of standard solution

A stock solution of glucose 100 $\mu\text{g/ml}$ was prepared in distilled water. Aliquots were taken from this solution to obtain sugar concentrations of 60 to 90 $\mu\text{g/ml}$. 1 ml of test solution was added and 1 ml of 5% phenol solution followed by 5 ml of concentrated

H_2SO_4 . The absorbance was measured after 10 min at 488 nm against blank (Sharma et al., 2012).

Preparation of test sample

10 mg of sample dissolved in 100 ml of distilled water. 1 ml of phenol solution was added; 1 ml of test solution was added, followed by 5 ml of concentrated H_2SO_4 . The absorbance was measured after 10 minutes at 488 nm against blank.

RESULTS AND DISCUSSION

Cytotoxic activity

The results of the cytotoxicity screened by MTT assay against MCF-7 and HUH7 cell lines of the Turmesac® is given in Figures 1 and 2. The formulated extract exhibited significant cytotoxicity against the MCF-7 cell line with IC_{50} value of 163.19 $\mu\text{g/mL}$ and does not show any cytotoxicity against the HUH-7 cell lines within the incubation period of 24 h in the concentrations ranging from 25 to 400 $\mu\text{g/mL}$, respectively. These findings suggest that the reduction observed in the viable cells following treatment with Turmesac® is due to cell death against the MCF-7 cell line. The leading cause of cancer is breast cancer, which holds fifth place in cancer-related deaths globally (Fitzmaurice et al., 2017). The high cost of chemotherapy for treating breast cancer and its side effects make this disease one of the most challenging (Blumen et al., 2016). The present observations provide preliminary data revealing that Turmesac® has potent cytotoxic activity against MCF-7 cells. The study serves as a scientific data for the use of Turmesac® chemotherapeutic properties as well as promising anticancer drugs.

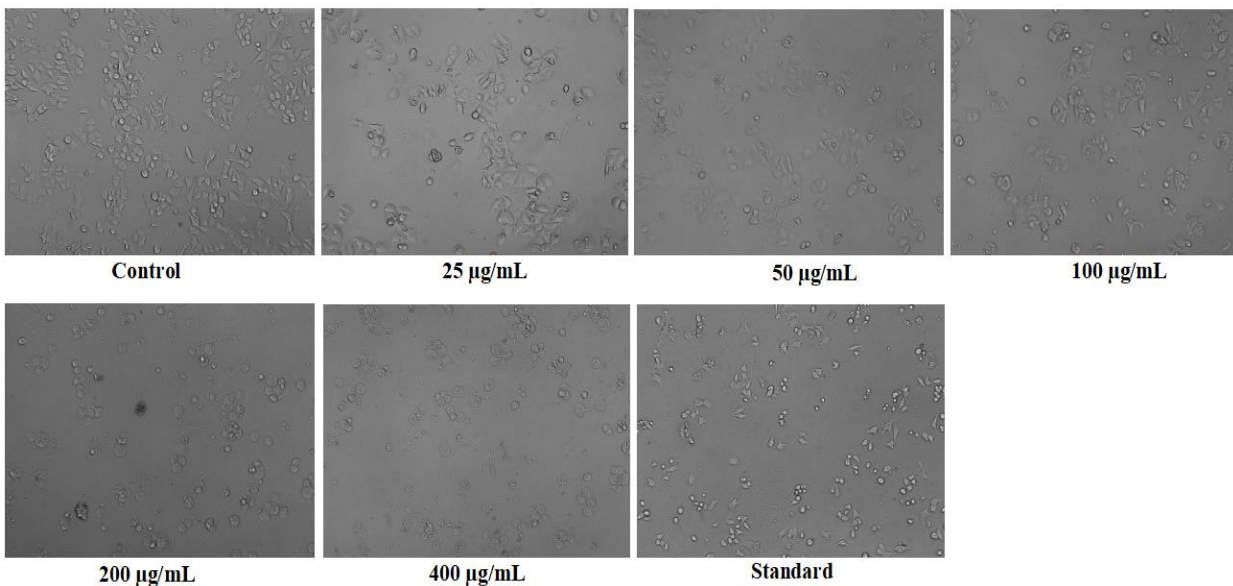


Figure 2. Direct microscopic observations of MCF-7 cells treated with Turmesac® visualized under inverted biological microscope with 10x magnification at the concentrations ranging from 25 to 400 µg/ml compared to Untreated control and standard Camptothecin.

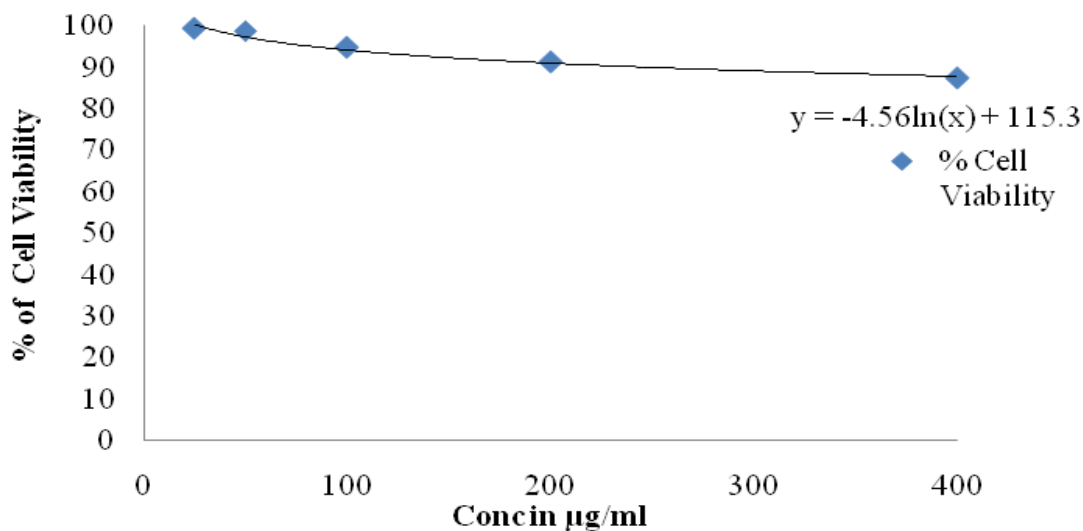


Figure 3. Cytotoxicity effect of Turmesac against HUH7 cell line after 48 h incubation.

Hepatoprotective activity

Hepatoprotective activity of the Turmesac® on HUH-7 cells from Human Liver cells was investigated *in vitro* 3-(4) 5-Dimethyl-thiazol-Zyl) - 2,5 biphenyl tetrazolium bromide (MTT) assay (Figures 3 and 4). In MTT assay, 25 to 400 µg/mL different concentrations were used by stimulating the cells with 200 µM of H₂O₂ for 24 h. The hepatoprotective activity of Turmesac® was evaluated using well-maintained HuH7 cells. H₂O₂ was used as

hepatotoxicant and silymarin was used as a standard positive control. The hepatoprotective activity of silymarin and Turmesac® were found to be IC₅₀ value 8.7 and 150.8 µg/mL, respectively. Shapiro et al. (2006) reported that curcumin showed the hepatoprotective effect and decreased levels of thiobarbituric acid reactive substances (TBARS), minimized oxidative stress and inhibited inducible nitric oxide (iNOS) protein and NF-κB in acute thioacetamide hepatotoxicity rats supplemented with 200 and 400 mg/kg per day curcumin. Curcumin

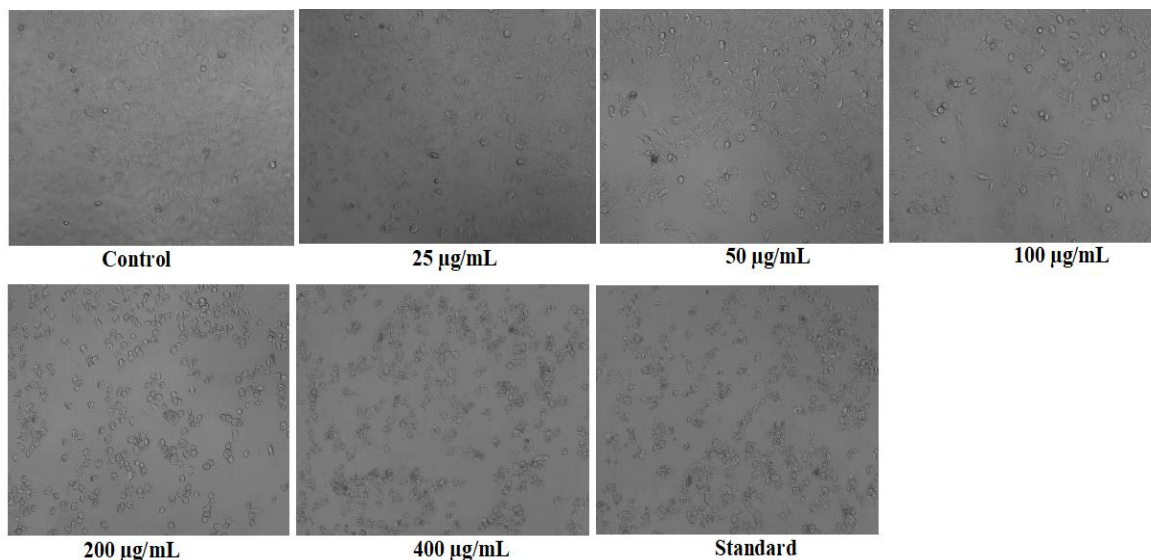


Figure 4. Direct microscopic observations of HUH7 cells treated with Turmesac visualized under inverted biological microscope with 10x magnifications at the concentrations ranging from 25 µg to 400 µg/ml compared to untreated control and standard Silymarin.

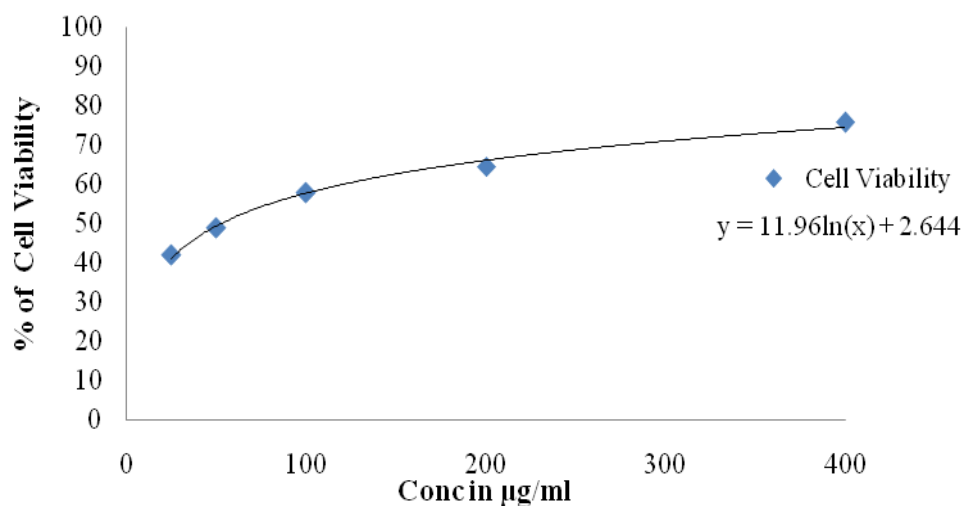


Figure 5. Cytotoxicity effect of Turmesac® vs H₂O₂ treated HUH7 cell line after 48 h incubation.

treatment shows hepatoprotective activity induced by *E. histolytica*, decreasing serum activities of ALT, ALP and γ -GTP, which were consistent with macroscopic and microscopic observations, suggesting that curcumin protects in both the early and late stages of liver infection (Deng et al., 2016). Several reports suggest that, the curcumin protects the liver against hepatotoxic compounds, such as carbon tetrachloride, alcohol and paracetamol (RVaratharajalu et al., 2016; Fernandez-Rojas et al., 2016). Turmeric is used as a traditional medicine and it has a therapeutic potential against jaundice and other disorders like liver, parasitic

infections, ulcers, various skin diseases etc. The rhizome juice from *C. longa* is very useful in the treatment of many diseases such as anthelmintic, asthma, gonorrhoea and urinary infections. Its essential oil is also used in the treatment of carminative, stomachic and tonic (Phansawan and Pongbangpho, 2007; Salama et al., 2013). The Turmesac® showed significant hepatoprotective activity against the HUH-7 cell lines by suppressing the toxic potential of H₂O₂ with increased concentrations of extract and ensuring the significant protective against the Human Liver Cell line (Figures 5 and 6).

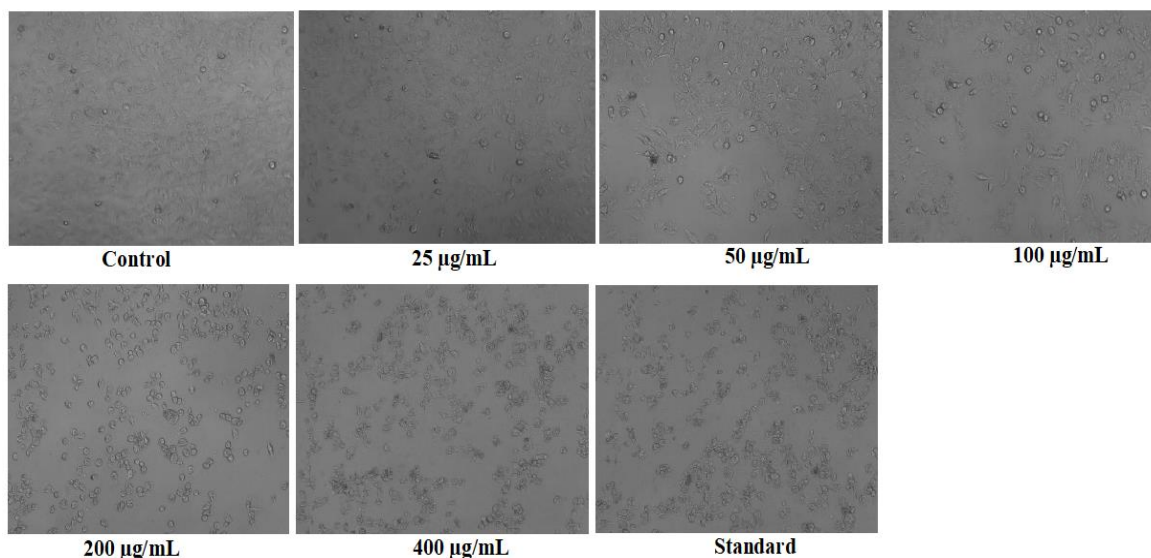


Figure 6. Direct microscopic observations of H_2O_2 stressed HUH7 cells treated with Turmesac® visualized under Inverted Biological microscope with 10x Magnification at the concentrations ranging from 25 μ g to 400 μ g/ml compared to untreated control and standard Silymarin.

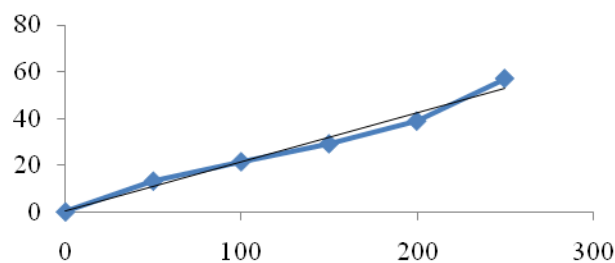


Figure 7. DPPH free radical scavenging activity (IC_{50} μ g/ml) of Turmesac®.

Table 1. DPPH free radical scavenging activity (IC_{50} μ g/ml) of Turmesac® and standard BHT.

S/N	BHT (μ g/ml)	OD (at 517 nm)	% inhibition of BHT	Sample concentration (μ g/ml)	OD (at 517 nm)	% of inhibition of Turmesac®	
1	Control	0.522	-	Control	0.493	0	
2	20	0.464	11.11	50	0.428	13.18	
3	40	0.367	29.69	100	0.387	21.5	
4	60	0.313	40.03	150	0.349	29.2	
5	80	0.273	47.7	200	0.301	38.94	
6	100	0.222	57.47	250	0.211	57.2	
			IC_{50} 82.65 μg/ml				IC_{50} 235.04 μg/ml

Antioxidant activity

The antioxidant activity of the Turmesac® is shown in Figure 7 and Table 1. The extract Turmesac® antioxidant

activity was compared with standard butylated hydroxytoluene (BHT). The free radical antioxidant scavenging activity of standard BHT and Turmesac® were found to be IC_{50} values of 82.65 and 235.04 μ g/ml,

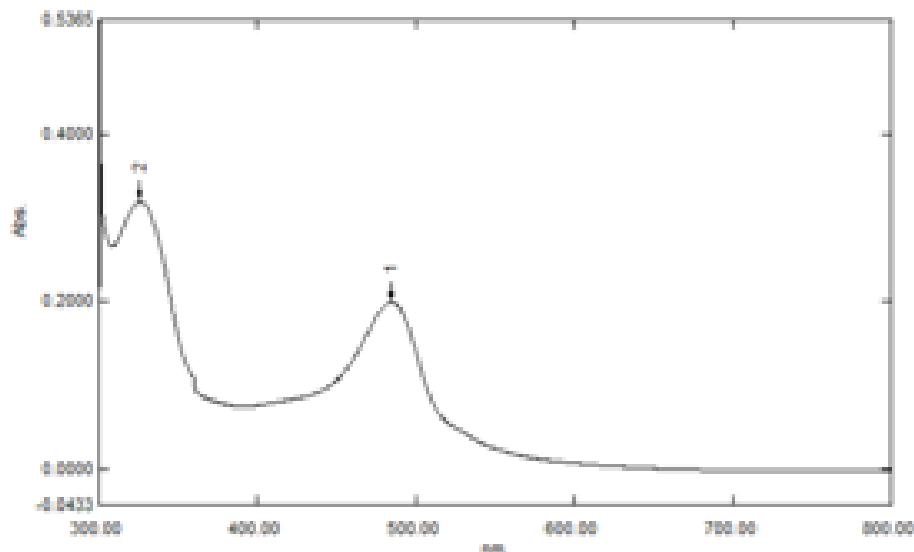


Figure 8. UV chromatogram of turmeric polysaccharides.

respectively. Antioxidants are tremendously important substances which possess the ability to protect the body from damage causing free radical induced oxidative stress. Free radicals are associated with many diseases. Hydroxyl, DPPH and superoxide radical scavenging activities are widely used to the quantitative determination of antioxidant capacities of biological samples and foods (Rahman and Islam 2013). The DPPH method was first introduced by Blois in 1958 and this method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. Most of the recent published papers are focused on the strategies to enhance the curcumin bioavailability. Its potent antioxidant properties are because of its chemical structure which consists of two methoxylated phenols that are connected by α , β unsaturated carbonyl groups (Priyadarsini, 2014). It has also been reported that the scavenging oxygen radical by GSH is related to the repair and neutralization of ROS induced damage (Nimse and Pal, 2015). One of these strategies is the preparation of curcumin complexes with natural compounds including piperine, or quercetin. For this reason, the study tried to prepare the turmeric polysaccharides to improve the antioxidant properties. The Turmesac® were screened for the presence of bioactive compound UV chromatogram showed the presence of polysaccharides (Figure 8).

Conclusion

The present investigation indicates that Turmesac® shows promising properties for antioxidant, cytotoxicity and hepatoprotective activities against MCF-7 and HUH-7 cells, respectively. The antioxidant, cytotoxic and

hepatoprotective role of Turmesac® is due to its polysaccharides. The study serves as a scientific data for the use of Turmesac® exhibited significant activity of antioxidant, cytotoxic and hepatoprotective drug.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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