J. Appl. Biosci., 51(2): 183-190, December, 2025
doi: 10.61081/joab/51v2i106

ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

RESEARCH ARTICLE

Evaluation of Antioxidant Properties and Cytotoxicity of Common Medicinal Plant Extracts Using Human Breast Cancer Cell-line (MCF-7)

Sejal Pal[1], Nidhi Patel[1], Nikhat J. Siddiqi[2], Chirag Prajapati[1], Ambika Arkatkar[1], Anjali Soni[1*] and Preeti Sharma[1*]
[1]Department of Biotechnology, Veer Narmad South Gujarat University, Udhna-Magdalla Road, Surat-395007, Gujarat, India
[2]Department of Medical Surgical Nursing, College of Nursing, PO Box 22452, King Saud University, Riyadh-11421, Saudi Arabia

ABSTRACT

Medicinal plants are accepted and approved for their therapeutic usage worldwide. The present study was carried out to screen medicinal plants for their antioxidant and cytotoxic activityusing MCF-7 cells. The methanolic leaf extracts of four plants viz., *Withania somnifera* (WS), *Moringa oleifera* (MO), *Stevia rebaudiana* (SR), *Nicotiana tabacum* (NT) have been used for the present study. Antioxidant activities determined using 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. Cytotoxicitywas determined using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. The antioxidant property of the extracts evaluated by DPPH assay show the activities in the following order NT > MO > SR >WS whereas antioxidant activity by ABTS assay was MO > NT > WS >SR. All the leaf extracts displayed cytotoxicity against MCF-7 cells in *in vitro* as well as *in silico* studies. The results suggest that the methanolic leaf extracts of the studied plants can be used as a source for exogenous antioxidants.

KEY WORDS: MCF-7 cells, leaf extracts, antioxidants, steroidal saponins, cytotoxicity, cancer, docking

INTRODUCTION

Globally, one fourth of the female cancer patients are reported to have breast cancer (Bray *et al*., 2018; Mohanraj *et al*., 2018) which can be identified into 3 categories based on hormone receptor (estrogen and progesterone) and Human Epidermal Receptor (HER2), hormone and HER2 positive, only HER2 negative and triple negative (Baharvand-Ahmadi *et al*., 2016). Breast cancer cells can have receptors for both or either estrogen or progesterone. These receptors allow them to use these hormones for growth. Chemotherapy targets to block these hormones or endocrine system. Many chemotherapeutic agents exert toxic side effects accompanied by reduced effectiveness due to development of resistance in the cancer cells (Ford *et al.,* 2016). The quest for bioactive products from plants and their extracts continue to be an active field of research despite of enormous contributions from synthetic chemistry (Peters *et al.,* 2002). Plant derived products have been reported to exert anticancer effects against animal tumor models and human cancer cell lines (Table 1) (Taylor, 2005). As per WHO, over the years 22% increase has been observed in use of medicinal plant among cancer patients. Many of the commonly available plants are known to possess antitumor properties (Orfali *et al*., 2016; Wang *et al*., 2016). Certain phytoconstituents have structural similarity with estrogen making them competent for binding with breast cancer cell receptors against these hormones. Hence, they can serve as an excellent antagonist for cancerous cells.

In the present study, we have used four medicinal plants viz., *Withania somnifera* (WS)*, Moringa oleifera* (MO)*, Stevia rebaudiana* (SR)*,* and *Nicotiana tabacum* (NT). The antioxidant potential of these medicinal plants was accessed by using DPPH and ABTS assays. The cytotoxicity of leaf extracts was studied on MCF-7 cells using the MTT and LDH assays along with molecular docking studies in relevance to Human Estrogen Receptor (PDB ID: 2IOK).

*Corresponding authors email: anjalisoni@vnsgu.ac.in, preetisharma@vnsgu.ac.in

© The Author(s). 2025 Open Access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) (https://creativecommons.org/licenses/by-nc-sa/4.0/)

Table 1: Plant species and traditional uses of the leaf extract which were screened for antioxidant and cytotoxic properties.

Family	Plant	Traditional uses
Solaneceae	*Withania somnifera*	Treatment of fever, tumours and ulcers (Taylor, 2005). Treat general debility, exhaustion, stress induced fatigue and insomnia Diarrhoea, dyspepsia and gastrointestinal disorders (Rodgman & Perfetti, 2008).
Moringaceae	*Moringa oleifera*	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling, blood pressure (Alam *et al*., 2011; Groark, 2010). Diarrheal dysentery, colitis, sores, skin infection, anaemia, cuts, scrapes, rashes, sign of aging (Debnath, 2008).
Asteraceae	*Stevia rebaudiana*	Sweetener used in medicinal green teas for treating heart burn and other ailments. Usually used for cavities, depression, diabetes, fatigue, heart support, hypertension, hyperglycaemia, infections, obesity, sweet cravings, tonic, urinary insufficiency, wounds (Gupta *et al*., 2017).
Solaneceae	*Nicotiana tabacum*	Treatment of toothache, respiratory complications and insect bites. Insecticide, anaesthetics, diaphoretics, sedatives, and emetic agents (Chen *et al*., 2018; Wagner *et al*., 1996).

MATERIALS AND METHODS

Plant materials and extraction

Leaves of *Withania somnifera* collected from a local nursery of Surat, *Moringa oleifera* from Veer Narmad South Gujarat University (VNSGU), Surat, and *Stevia rebaudiana* and *Nicotiana tabacum* collected from local farmers of Vadodara, Gujarat. These plants were authenticated by Dr. M.N. Reddy (Ex- Professor, Department of Biosciences, VNSGU, Surat). The voucher specimens–BVBRC1206, BVBRC1207, BVBRC1208, and BVBRC1209 deposited at Shri Bapalal Vaidhya Botanical Research Centre, VNSGU, Surat. Collected plant leaves were washed and rinsed with distilled water, shade dried, powdered and subjected to Soxhlet extraction method (Wagner *et al*., 1996). Dried powder 5 g from each sample was extracted using 150 ml of methanol. The methanolic extract was then concentrated using heating mantle, weighed and re-dissolved in known amount of methanol to a final concentration of 20mg/ml. Each plant extract was filter-sterilized using 0.2µm filter and stored at 4ºC.

Preliminary phytochemical analysis

The extracts were analyzed for the presence of various phytochemicals using standard screening procedures (Horiuchi *et al.,* 1988). Conventional protocols were used for detection of alkaloids, flavonoids, glycosides, steroids, saponins etc.

Cell culture

Human breast cancer MCF-7 cell line was obtained from National Centre for Cell Sciences, Pune, India. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (HiMedia, Mumbai, India), 10% Fetal Bovine Serum (FBS), 1% penicillin, and 1% streptomycin. Cells were washed by phosphate buffer saline (PBS) and harvested by trypsinization and were plated in 96 well plates (5×10^4cells/well) and incubated under 5% CO2 and at 37^oC for 24 hours.

In vitro antioxidant assays

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assay: Stock solution of $ABTS^{\cdot *}$ was prepared by mixing 10 ml of 7 mM $ABTS^{\cdot *}$ solution with 10 ml of 2.4 mM potassium per-sulfate solution and left it in dark at room temperature for 16 hours. Free radical scavenging activity was assessed for various concentrations of extracts (25, 50, 100 and 200µg/ml) by mixing with 3.0 ml of ABTS working standard. The decrease in the intensity of blue color of $ABTS^{\cdot}$* to colorless solution indicated the scavenging potential of different plant extracts. The $ABTS^{\cdot}$* scavenging capacity of the extracts was compared with a standard viz., butylatedhydroxytoluene (BHT).

$$\% \text{ Scavenging} = [(A_0 - As)/A_0] \times 100$$

Where, A_0= O.D. control and A_S= O.D. plant leaf extract.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay : DPPH assay was carried out according to Re *et al.* (1999). About 100µl of various extracts at different concentrations (25, 50, 100 and 200µg/ml) were mixed with three ml of DPPH (2 mM) in methanol. The

reaction mixtures were incubated for 30 min in dark and the absorbance was measured at 517 nm.

% Scavenging = $[(A_0 - As)/A_0] \times 100$

Where, A_0 = O.D. control and A_S = O.D. plant leaf extract.

In vitro Cytotoxicity Assays

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay : MTT assay was performed according to Horiuchi *et al.* (1988). For MTT assay, MCF-7 cells were plated in 96-well platewith cell density 5×10^3 to 10^4 cells/well. The cells were then exposed to 6 different concentrations (6.25, 12.5, 25, 50, 100, 200µg/ml) of each extract for 24 hours. After incubation with the drug, 10 µL of the MTT reagent prepared in 5.0 mg/ml phosphate buffered saline (PBS) was added to each well, and incubated for 4 hours at 37°C. The MTT crystals were then solubilized by adding 100ìl of the Sorenson Glycine buffer. Absorbance was measured at 490 nm using a micro plate reader (BioTek® Instruments Inc.).

%Cell Viability = O.D. sample / O.D. control × 100

% Cytotoxicity = 100 – % Cell viability

Lactate Dehydrogenase (LDH) Assay : LDH assay was performed as per the protocol (HiMedia EZ count™). The MCF-7 cells (5×10^3 to 10^4cells/well) were grown overnight at 37°C on 96-well flat bottom cell culture plates, then exposed to 6 different concentrations (6.25, 12.5, 25, 50, 100, 200 µg/ml) for 24 h. After incubation, 50 µl of supernatant from each well was transferred in a fresh 96-well plate and 50 µl LDH reagent was added in each well the plate was wrapped with aluminum foil and incubate for 2 hours at 37º C in a 5 % CO_2 environment. 50 µl of stop solution (1M acetic acid) was added in each well, swirled and absorbance was measured at 490 nm in microplate reader and % cytotoxicity was calculated as per following formula:

% cytotoxicity = Experimental- background control/ maximum LDH control – background control

In-silico Analysis

Retrieval of protein and ligands from database : Three-dimensional (3D) structure of Human estrogen receptor, alpha ligand-binding domain is a complex with compound 1D having PDB code 2IOK,was downloaded from Brookhaven Protein Data Bank (PDB) (http://www.rcsb .org/). The structure of all the phytochemicals in this study were retrieved from IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics database.Total 54 leaf compounds (ligand) of two plants under study viz. *M. oleifera* (31) and *S. rebaudiana* (23) were downloaded from database for *in-silico* analysis.

Table 2: Preliminary phytochemical analysis of leaf extracts of various medicinal plants

Metabolites	*Withania*	*Moringa*	*Stevia*	*Nicotiana*
Alkaloid	+	+	+	+
Glycosides	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Steroids	-	+	+	-
Terpenoids	+	+	+	+
Coumarins	+	+	+	+
Saponins	+	-	+	-
Phenol	-	+	-	+
Triterpene	-	+	+	+
Phlobatannins	+	+	-	+
Antho- & beta cyanins	+	+	-	-
Flavanoids	+	-	+	+
Quinone	-	-	-	-
Oxalate	-	-	-	-
Emodins	-	-	-	-
Carboxylic acid	-	-	-	-

+ present; - absent

Table 3: DPPH and ABTS- radical scavenging activities of various methanolic leaf extracts

Extract	Conc. (µg/ml)	% Inhibition by DPPH Assay	% Inhibition by ABTS Assay
Withania somnifera	25	4.55	30.88
	50	4.55	30.03
	100	5.50	33.95
	200	7.94	41.46
Moringa oleifera	25	5.93	28.15
	50	6.67	31.39
	100	7.09	41.97
	200	10.06	57.16
Stevia rebaudiana	25	4.76	5.63
	50	4.97	16.72
	100	7.41	21.33
	200	9.74	29.01
Nicotiana tabacum	25	5.61	20.47
	50	10.91	20.47
	100	11.01	34.81
	200	17.16	48.46

Table 4: Binding affinity and residues involved in interaction with target protein

Target Protein	Sample	Ligands	Binding Affinity (Kcal/mol)	Residue involved in interactions
(PDB ID: 2IOK)	*Stevia rebaudiana*	Rebaudioside A	**-8.3**	LEU-1349, PRO 1325, GLU-1353, LYS-1449, PRO-1324, PHE-1445, GLU-1323, GLU-1443, GLN-1441, ASN-1439, MET-1396, GLU-1397, LEU-1440, GLY-1442, HIS-1398, LEU-1403, ARG-1394, TRP-1393, ILE-1326, PRO-1406, ALA-1405, LEU-1327
	Moringa oleifera	Apigenin	**-9.2**	ARG-394, PHE-404, LEU-391, ALA-350, LEU-346, LEU-525, THR-347, LEU-387
		Hesperetin	**-9.2**	MET-343, LEU-525, ARG-394, THR-347, ALA-350, LEU-387, PHE-404, LEU-391

Preparation of macromolecules : The X-ray crystallographic 3D structure of protein target having PDB ID: 2IOK (Fig- 6) was retrieved through RCSB Protein Data Bank (https://www.rcsb.org/) and visualized by Rasmol (Tian *et al.,* 2018; Vivek-Ananth, 2023). Water molecules, ligands and other hetero atoms were removed from the protein molecules. Hydrogen atoms were added to protein targets for correct ionization and tautomeric states of amino acid residues.

Analysis of target active binding sites : Active sites of the target were identified by CASTp server (Sayle & Milner-White *et al.,* 1995). CASTp (Computed Atlas of Surface Topography of proteins) is used to verify the binding sites of a protein. It includes annotated functional information of specific residues on the protein structure (Tian *et al*., 2018).

Molecular docking studies : A computational ligand-target docking approach was used to analyze structural complexes of the target protein with selected compounds. The docking studies were performed by using PyRx (Autodock vina) tools (http://pyrx.sourceforge.net/downloads). Detailed visualization and comparison of the docked sites of target proteins and ligands was done using Discovery Studio visualizer (http://www.accelyrs.com).

Data analysis : All data were expressed as means ± standard deviation (SD). Statistical comparisons of the results were made by using one-way analysis of variance (ANOVA). P value <0.05 was considered significant.

RESULTS, DISCUSSION AND CONCLUSION

Bioactivity studies

Qualitative phytochemical analysis has shown the presence of alkaloids, glycosides, carbohydrates, proteins etc. in all the methanolic plant extracts (Table 2). Studies of Alam *et al.* (2011) in which they reported the presence of high concentration of polyphenols in Moringa, as well as phytochemicals like sterols, terpenoids, flavonoids,

Table 5: Binding affinity obtained through docking with Human estrogen receptor alpha ligand-binding domain in complex with compound 1D (PDB ID: 2IOK) against *Moringa oleifera*

Ligands	Binding Affinity (Kcal/mol)
(+)-Gallocatechin	-7.9
(-)-Epicatechin	-8.4
1,7-Octadien-3-ol, 2,6-dimethyl-	-5.5
4-(Rhamnosyloxy) phenylacetonitrile	-7.1
4-Hydroxycinnamic acid	-6.4
alpha-Carotene	-9.1
Apigenin	**-9.2**
Ascorbic acid	-5.2
Caffeic acid	-6.4
Chlorogenic acid	-8.3
Cianidanol	-8.4
Ellagic acid	-8.7
Epigallocatechin	-8.8
Ethyl palmitate	-5.9
Ferulic acid	-6.4
Gallic acid	-6.1
Gossypetin	-8.6
Hesperetin	**-9.2**
Luteolin	-8.8
Myricetin	-8.5
N,alpha-L-rhamnopyranosylvincosamide	-8.2
Niazimicin A	-6.6
Niaziminin B	-7.5
Niazinin	-7.2
Niazirinin	-7.8
Palmitic acid	-5.7
Phenylacetonitrile	-5.6
Quercetagetin	-8.4
Rutin	-8.3
Trigonelline	-5.1
Vanillin	-5.5

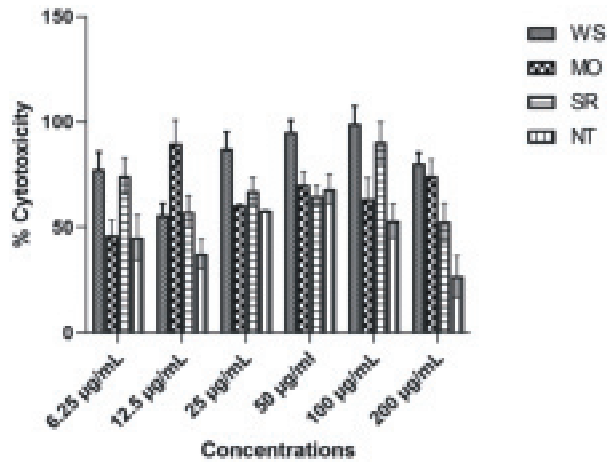


Fig.1: Cytotoxicity of various leaf extracts by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

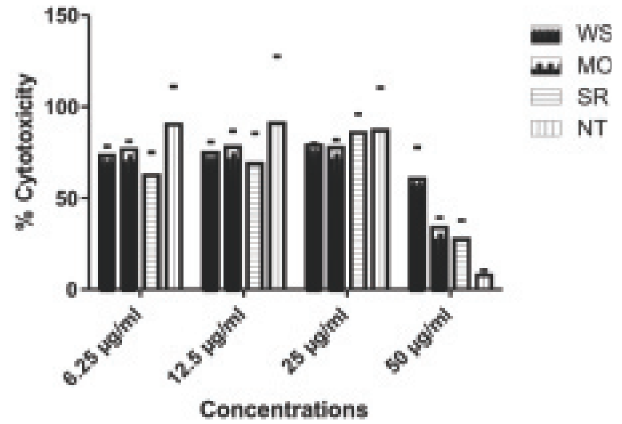
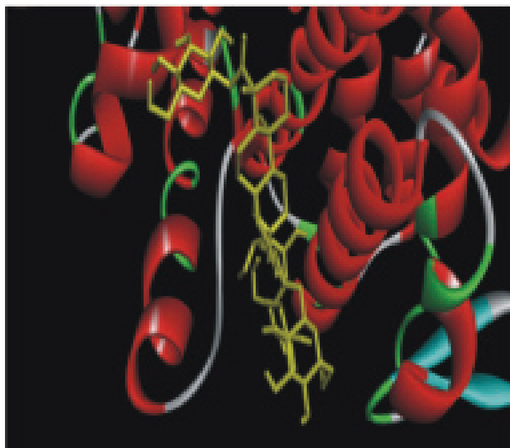


Fig. 2: Cytotoxicity of various leaf extracts by Lactate Dehydrogenase (LDH) Assay.



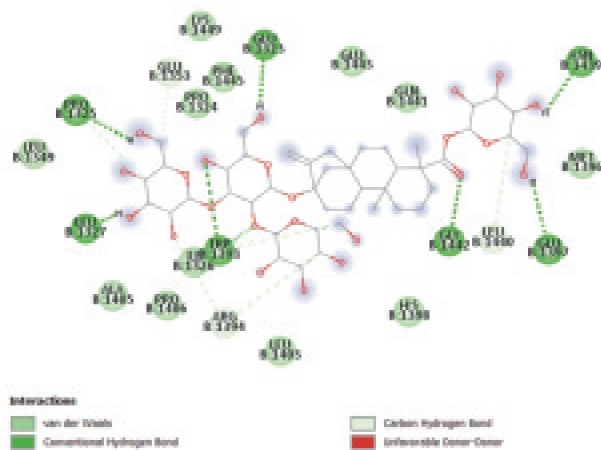
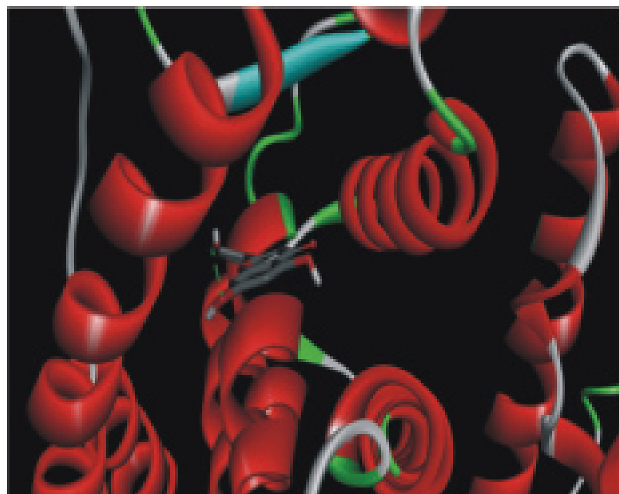


Fig. 3: Rebaudioside A docked with target protein (PDB ID: 2IOK)



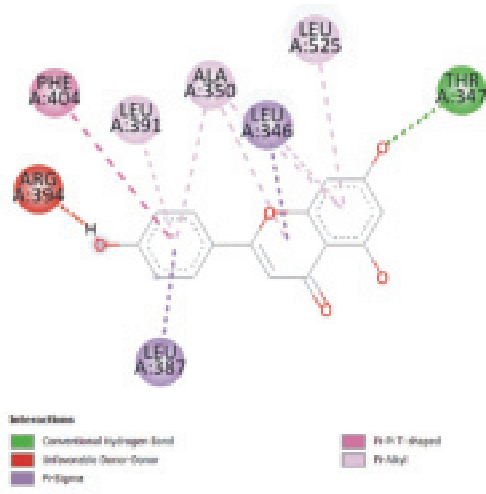
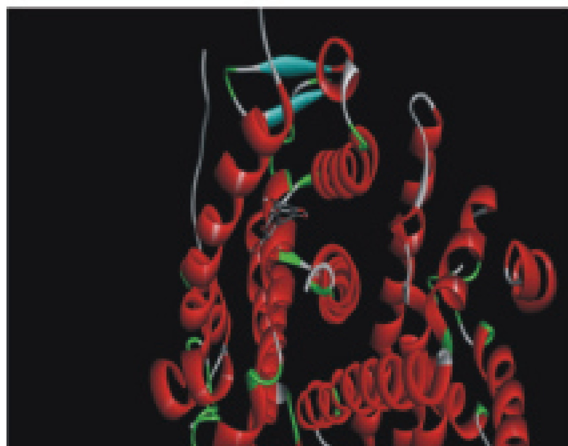


Fig. 4: Apigenin docked with target protein (PDB ID: 2IOK)



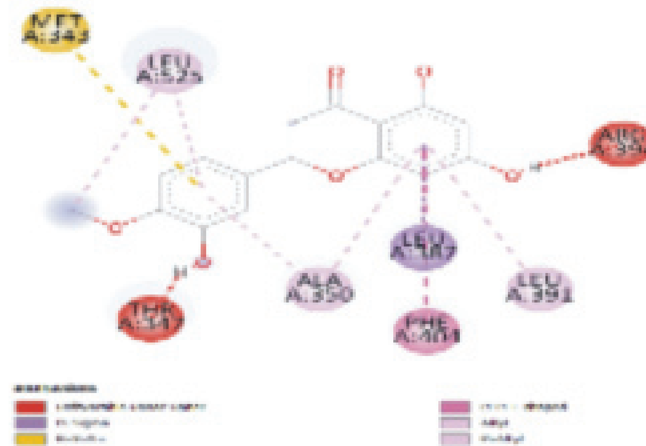


Fig. 5: Hesperetin docked with target protein (PDB ID: 2IOK)

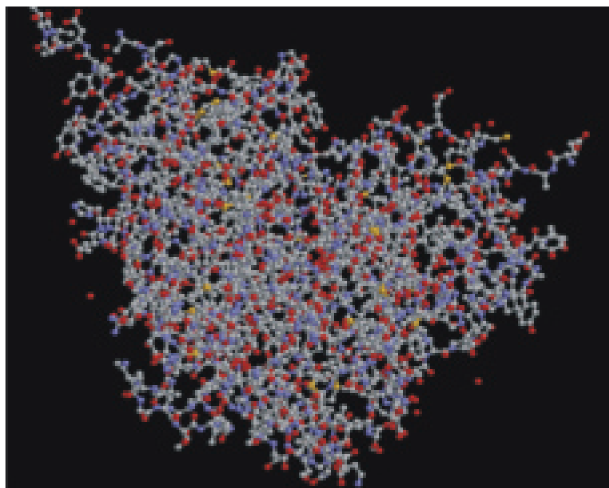


Fig. 6: Structure of 2IOK

saponins and reducing sugars etc. in the leaf extract (Rodgman & Perfetti, 2008). Terpenes, saponins, flavonoids, terpenoids and glycosides present in WS have been reported to be responsible for anticancer properties (Groark, 2010). In the present study however, terpenes were not detected which may be attributed to (Dania *et al*., 2014; Stohs & Hartman, 2015). Variations occur due to the factors such as genetic, soil, climate, time of harvest, and storage conditions (Debnath, 2008). SR is commonly known as sweet-leaf. The leaves of SR have been reported to contain flavonoids, phenolic acids, fatty acids, proteins and vitamins, which are responsible for its various biological activity (Gupta *et al*., 2017). The methanolic leaf extract of SR showed detectable presence of alkaloids, flavonoids, glycosides, etc. (Table 2) (Valadez-Vega *et al.,* 2013). The lack of detectable phenols in the SR leaves

Table 6: Binding affinity obtained through docking with Human estrogen receptor alpha ligand-binding domain in complex with compound 1D (PDB ID: 2IOK) against *Stevia rebaudiana*

Ligands	Binding Affinity (Kcal/mol)
Octanal	-4.6
3-Methylbutanal	-3.7
1-Penten-3-OL	-3.8
Nonanal	-4.6
2-Heptenal	-4.4
1-Octen-3-OL	-4.7
2,4-Heptadienal	-4.5
2-Ethylhexanol	-4.8
Hexanal	-4.0
Decanal	-4.9
Methyl vinyl ketone	-3.9
Heptanal	-4.3
Pentanal	-3.8
1-Octene	-4.4
1-Pentanol	-4.0
2-Hexenal	-4.2
alpha-Pinene	-5.7
Tetrahydrofuran	-3.6
beta-Caryophyllene	-7.6
Limonene	-6.3
1-Heptene	-4.3
4-Hydroxy-2-butanone	-3.8
Rebaudioside A	**-8.3**

may be attributed to the extraction with methanol. NT leaf extract showed the presence of glycosides, carbohydrates, proteins etc. (Table 2).

Antioxidant potential of all the extracts was analyzed using DPPH and ABTS free radical scavenging assays (Table 3). The antioxidant activity of the extracts using DPPH assay showed the following order NT > MO > SR > WS. Similarly, the antioxidant activity using ABTS showed slightly different trend MO > NT > WS > SR. The antioxidant activity in the leaves of MO may be attributed to the presence of polyphenols, phenolic acids, flavonoids, glucosinolates, and alkaloids (Debnath, 2008). The presence of phenols, flavonoids etc. may have contributed to the antioxidant properties of these leaf extracts.

Cell cytotoxicity of leaf extracts was analyzed by MTT and LDH assays (Figs. 1, 2). The leaf extract of SR showed concentration dependent cytotoxicity towards MCF-7 cells. Chen *et al*. (2018) reported that tabsesquiterspene A (1) present in NT, found to be a major contributor for its cytotoxic effect against cancer cells (Mahavorasirikul *et al*., 2010). In the present study also, NT resulted cytotoxicity against cancer cells using both MTT and LDH assay. Cytotoxicity observed MO leaf extract provided better result at lower concentration and the effects could be attributed to phytoconstituents present in MO (Chen *et al*., 2018). All the extracts showed more than 50% cytotoxicity at 6.25µg concentration in LDH assay. WS and NT showed cytotoxicity in both the assays but they were inconsistent as compared to the effect of MO and SR extract. There is a need of further evaluation on the active components of MO and SR extracts for their chemotherapeutic properties which may lead to the development of anticancer drug(s).

Docking studies

Breast cancer cells have affinity towards estrogen and progesterone hormone; these hormones act along with oncogenes facilitating growth of these cancer cells. Various studies have shown that most of the phytochemicals do exhibit structure similarity with estrogen resulting in competitive binding against estrogen. In the present docking study role of these phytochemicals as an antagonist against cancer cells was studied. Human estrogen receptors that are present on the surface of breast cancer cells were selected as a target protein (Liang *et al*., 1998). Total 54 leaf compounds of two plants under study viz. *M. oleifera* (31) and *S. rebaudiana* (23) were downloaded from database to evaluate their cytotoxic activity.

These compounds were docked at the catalytic site of the Human estrogen receptor alpha ligand-binding domain (PDB ID: 2IOK). The top scorers were two compounds (Apigenin and Hesperetin) with maximum binding energy -9.2 kcal/mol with the target protein in *Moringa oleifera*. Similarly, in *Stevia rebaudiana* top scorer with binding energy -8.3 kcal/mol found in Rebaudioside A. Docked pose of these compounds with the target protein have been presented in Figs. 3-5. Thus from the present study it can conclude about H-bond interactions with the active site residues, estimated free energy of binding and residues involved in interactions as summarized in Table 4. Molecular docking studies shows that the identified compounds from *M. oleifera* and *S. rebaudiana* has the potential to inhibit target protein (PDB ID: 2IOK) which indicates its high cytotoxic activity have been given in Table 5,6. The results of the study suggests that the methanolic leaf extracts of the plants viz., MO and SR have potent bioactive compounds which could be utilized as anti-cancer agents in future.

Declaration of interest statement

No conflict of interest is noted.

Funding

This work was funded by the Major/Minor Research Project (No. UGC/17162/2023), Veer Narmad South Gujarat University, Surat, Gujarat, India to Dr. Preeti Sharma.

REFERENCES

Alam, N., Hossain, M., Khalil, M.I., Moniruzzaman, M., Sulaiman, S.A., & Gan, S.H. (2011). High catechin concentrations detected in *Withania somnifera* (ashwagandha) by high performance liquid chromatography analysis. *BMC Comple. Altern Med.* , 11(65): 1-8. doi: 10. 1186/1472-6882-11-65.

Baharvand-Ahmadi, B., Bahmani, M., Tajeddini, P., Rafieian-Kopaei, M., & Naghdi, N. (2016). An ethnobotanical study of medicinal plants administered for the treatment of hypertension. *J. Renal Inj. Prev*., 5(3): 123-128. doi: 10.15171/jrip.2016.26.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R., Torre, L., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin*., 68(6): 394-424. doi: 10.3322/caac.21492.

Chen, J., Xia, Y., Sui, X., Peng, Q., Zhang, T., Li, J., & Zhang, J. (2018). Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. *Oncotarget*., 9(41): 26299-26308. doi: 10.18632/oncotarget.25233.

Dania, S.O., Akpansubi, P., & Eghagara, O.O. (2014). Comparative effects of different fertilizer sources on the growth and nutrient content of moringa (*Moringa oleifera*) seedling in a greenhouse trial. *Ad. Agricul*., 726313: 1-6. doi: 10.1155/2014/726313.

Debnath, M. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. *J. Med. Plant. Res*., 2: 45-51.

Ford, C.T., Richardson, S., McArdle, F., Lotito, S.B., Crozier, A., & McArdle, A. (2016). Identification of (poly) phenol treatments that modulate the release of pro-inflammatory cytokines by human lymphocytes. *Br.J.Nutr*., 115(10): 1699-1710. doi: 10.1017/S0007114516000805.

Groark, K.P. (2010). The angel in the gourd: ritual, therapeutic, and protective uses of tobacco (*Nicotiana tabacum*) among the tzeltal and tzotzil maya of Chiapas, Mexico. *J. Ethnobiol*., 30(1): 5-30.

Gupta, E., Kaushik, S., Purwar, S., Sharma, R., Balapure, A.K., & Sundaram, S. (2017). Anticancer Potential of Steviol in MCF-7 Human Breast Cancer Cells. *Pharmacogn Mag*., 13(51): 345-350.

HiMedia Laboratories. EZcount™ Lactate Dehydrogenase (LDH) Cell Assay Kit (CCK036).

Horiuchi, N., Nakagava, K., Sasaki, Y., Minato, K., Fujiwara, Y., & Nezu, K. (1988). *In vitro* antitumor activity of mitomycin C derivative (RM49) and a new anticancer antibiotic (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. *Cancer Chemother Pharmacol.,* 22(3): 246-50.

Liang, J., Edelsbrunner, H., & Woodward, C. (1998). Anatomy of protein pockets and cavities: measurement of binding site geometry and implications for ligand design. *Protein Sci.*, 7(9): 1884-1897. doi: 10.1002/pro.5560070905.

Mahavorasirikul, W., Viyanant, V., Chaijaroenkul, W., Itharat, A., & NaBangchang, K. (2010). Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro*. *BMC Complement Altern Med*., 10: 55. doi: 10.1186/1472-6882-10-55.

Mohanraj, K., Karthikeyan, B.S., & Vivek-Ananth, R.P. (2018). IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. *Sci. Rep.,* 8: 4329. doi: 10.1038/s41598-018-22631-z.

Orfali, G.C., Duarte, A.C., Bonadio, V., Martinez, N.P., de Araújo, MEMB, & Priviero FBM. (2016). Review of anticancer mechanisms of Isoquercitin. *World J. Clin. Oncol*., 7(2): 189-199. doi: 10.5306/wjco.v7.i2.189.

Peters, G.J., Backus, H.H., Freemantle, S., Van Triest, B., Codacci-piasanelli, G., & Van Der Wilt CL. (2002). Induction of thymidylate synthase as a 5-fluorouracil resistance mechanism. *Biochim. Biophys Acta*, 1587(2-3): 194-205. doi: 10.1016/s0925-4439(02)00082-0.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*., 26(9-10): 1231-1237. 10.1016/S0891-5849(98)00315-3.

Rodgman, A., & Perfetti, T.A. (2013). The Chemical Components of Tobacco and Tobacco Smoke (2nd ed.). CRC Press. https://doi.org/10.1201/b13973.

Sayle, R.A., & Milner-White, E.J. (1995). RasMol: biomolecular graphics for all. *Trends biochem Sci*., 20(9): 374.

Stohs, S.J. & Hartman, M.J. (2015). Review of the Safety and Efficacy of *Moringa oleifera*. *Phytother Res.,* Jun, 29(6): 796-804.

Taylor, L. (2005). The Healing Power of Natural Herbs. Garden City Park, NY: Square One Publishers, Inc. pp. ISBN 0-7570- 0144-0. http://rain-tree.com/stevia.htm.

Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res.*, 46(W1): W363-W367.

Valadez-Vega, C., Delgado-Olivares, L., A., J., Alans, E., Villagomez Ibarra, J.R., Ramrez, E., & Caldern, Z. (2013). The Role of Natural Antioxidants in Cancer Disease. *InTech.* doi: 10.5772/51503

Vivek-Ananth, R.P., Mohanraj, K., Sahoo, A.K., & Samal, A. (2023). IMPPAT 2.0: An Enhanced and Expanded Phytochemical Atlas of Indian Medicinal Plants. *ACS Omega,* 8(9): 8827-8845. doi: 10.1021/acsomega.3c00156.

Wagner, H. & Bladt, S. (1996). Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edition, Springer-Verlag, Berlin. doi: 10.1007/978-3-642-00574-9