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In Vitro Callus Induction, Phytochemical Screening, and *In Silico* Docking Of *Vitex Negundo* Secondary Metabolites against HER2 Protein, A Breast Cancer Biomarker

Kumaresan Kowsalya[1], Jaganathan Sakthi Yazhini Preetha[1], Perakasam Lalitha Jemima Aashmi[2], Sivakumar Kowsalya [3], Muthukrishnan Arun[1]*

[1]*Department of Biotechnology, Bharathiar University, Coimbatore-641046, Tamil Nadu, India*

[2]*Department of Integrative Biology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore - 632 014, Tamil Nadu, India*

[3]*PG and Research Department of Biotechnology, Nandha Arts and Science College, Erode-638052, Tamil Nadu, India*

[*]*Corresponding Author Email ID: arun@buc.edu.in*

Abstract

Among different cancer types, about 20 % of breast cancer overexpress and amplifies multiple copies of human epidermal growth factor receptor (HER2) oncogene. HER2 plays a major role in tumour development and progression in breast cancer. Successful treatment in women is achieved with HER2-directed therapy by using an anti-HER2 antibody which implies the significance of the HER2 protein. Hence the identification of plant-based inhibitors for HER2 protein will be a sustainable, side effect-free, and cost-effective strategy for breast cancer treatment. Moreover, *Vitex negundo* is a multipotent medicinal plant often called as “Sarvaroga nivarani” remedy for all diseases. It belongs to the family *Verbenaceae.* This plant is rich in secondary metabolites with a wide spectrum of therapeutic properties such as antimicrobial, anti-inflammatory, astringent, bronchodilator, anti-depressant, and hepatoprotective. It has an immense role in balancing hormonal fluctuations in women. This beneficial therapeutic role of *Vitex* can be utilized in terms of breast cancer. Further, we intend to unravel its anticancer potential in breast cancer through preliminary *in silico* studies. In this study, we selected 29 compounds present in the methanolic extract of *Vitex negundo* from the literature and molecular docking studies were done by using HER2 as the target protein. Our *in silico* results revealed that D-Glucose, 6-O-D-galactopyranosyl compound has the highest effective binding energy and was found to be a potential inhibitor for HER2 protein. We also conducted *in vitro* studies to establish a better callus induction protocol for *Vitex negundo* with a combination of 2,4-D (1 mg/L) and IAA (0.5 mg/L) in MS solid medium. It produced the highest percentage of callus response (81.2%) and callus biomass (fresh weight=68.3±0.02mg; dry weight=17.2±0.03mg). Leaf-derived callus has shown elevated levels of total polyphenolic (40 ±0.05 mg GAE/g of extract) and flavonoid (25±0.05 mg QE/g of extract) contents as compared to control, as well as higher inhibition percentage (80.2%) and ferrous chelating activity (75.9%). Thus, establishing plant tissue culture in *Vitex negundo* can serve as a perpetual and potent source for the large-scale production of pharmaceutically important secondary metabolites in cancer treatment.

Keywords: *Vitex negundo*, HER2, docking, callus

Introduction

Vitex negundo L. is a potent aromatic medicinal plant that belongs to the family *Verbenaceae.* It is widely distributed in India This plant is rich in phytoconstituents such as vitexin, isovitexin, orientin, isoorientin, acubin, agnuside and negundoside (Umamaheswari, 2012). In India, the traditional medicine system depicts *Vitex negundo* as ‘*sarvaroga nivarani*’ which means remedy for all diseases (Suganthi, 2016). etc. It is also used as a repellent, insecticide, and larvicidal. Leaf extract is employed as a nervine tonic, tranquilizer, and vermifuge. The common name of *Vitex negundo* includes Chinese chaste tree, horseshoe vitex, monk’s pepper, and five-leaved chaste trees It is native to tropical eastern and southern Africa and Asia. It is used as folk medicine in South & Southeast Asia. The leaves have five leaflets in a palmate arrangement, which are lanceolate, 4–10 cm long, hairy beneath, and pointed at both ends (Sharma*,* 2014). It prefers light well-drained loamy soil in a warm sunny position sheltered from cold drying winds and succeeds in poor dry soils. It can tolerate temperatures of about -10°C. It also produces root suckers, and thus is used for planting against soil erosion and for afforestation, especially in the stabilization of forest lands affected by floods. It is used as a food crop and also as a source of timber. A large aromatic shrub, the plant is distributed throughout the greater part of India up to an altitude of 1500 m in the outer Himalayas (Bhasri et al, 2014; Bhosale et al, 2016). *Vitex negundo,* locally known as ‘Nirgundi/Sindhvar’ is an important medicinal plant and is used for the treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally

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validated. A number of pharmacological activities have been attributed to *Vitex negundo* such as enzymes inhibition, nitric oxide scavenging activity, snake venom neutralization activity, antifeeding activity, antiradical and anti-lipoperoxidative, CNS activity, hepatoprotective activity, antibacterial activity, anti-fungal, larvicidal activity, antiandrogenic effects, mosquito repellent activity etc., (Tasduq, 2008). Fresh leaves of *Vitex negundo* yield 0.05 percent essential oils. Air-dried leaves yield alkaloid and two new iridoid-glycosides viz – nishindaside and negundoside (Vimal *et al.,* 2011). *Vitex* leaf extract showed anti-cancer activity against *Ehrlich ascites* tumor cells (Ahmad, N., & Anis, M. 2007). In plant tissue culture, callus tissue is an essential material in plant cell culture systems. Callus is a de-differentiated state of tissue through the exogenous application of plant growth hormones *in vitro*. Different physiological and morphogenic responses can also be observed through callus culture like somaclonal variations, somatic embryogenesis, and organogenesis. It can also paves the way for isolating economically valuable phytochemicals, which can avoid the collection of plant materials from natural wild sources (Flick, 1983; Raghavan, 1986; Larkin and Scowcraft, 1981).

Overexpression of human epidermal growth factor receptor 2 (HER2) plays an important role in the development and progression of cancer and it is a novel therapeutic target for breast and ovarian cancer. It is reported that the purified phytocompounds present in the *Vitex negundo* perturbs cancer signaling pathways and induces apoptosis in breast cancer cell lines (Zhou et al, 2009).

Hence, the aim of this present study is to identify HER2 inhibitory compounds through docking studies and *in vitro* optimization of callus cultures in *Vitex negundo.* The main objectives are (1) Identification of HER2 inhibitory compounds present in the leaf extracts of *Vitex negundo* through *in silico* studies, (2) *In vitro* optimization of callus cultures of *Vitex negundo* for enhanced production of bioactive compounds, (3) To analyze phytochemicals present in methanolic extract of *Vitex negundo* through qualitative and quantitative analysis (4) To compare the antioxidant activity of methanolic extract prepared from *Vitex negundo* leaves (control) and *in vitro* callus induced on solid MS medium.

Materials and methods:

Molecular docking of selected *Vitex* secondary metabolites with HER2 target protein:

Collection of ligand and target protein:

Target protein: HER2 Protein (PDB ID:3PPO) was used for this study and the crystal structure was downloaded from Protein Data Bank. A ligand set of 29 phytocompounds from methanolic extract of *Vitex negundo* leaves was identified from Kumar et al, 2010 and downloaded from the PubChem database. Ligand preparation was done by Ligprep module. Phytocompounds were allowed to dock with HER2 Protein using auto dock.

In vitro callus induction:

Plant sourcing and preparation of explants:

Vitex negundo plants were collected from Kerala Agricultural University (KAU). These plants were planted in the experimental garden at the Department of Biotechnology, Bharathiar University, Coimbatore. It has been authenticated in the Botanical Survey of India, South Regional Center, Coimbatore. Healthy young leaves of three months old *Vitex negundo* L. were used as explants. The selected explants were washed under running tap water followed by treatment with a surfactant, Tween 20 (5% v/v) for 10 min. It was further treated with 70% ethanol for 10-15 seconds, followed by a double distilled water wash. Then the explants were sterilized with mercuric chloride (0.1% w/v) solution for 2-3 minutes and were finally rinsed with sterile distilled water for three times. The leaf disk was prepared for about 5mm-8mm (Jawahar.,2008). Young leaf explants were inoculated on MS medium containing 0.3% sucrose, 0.7% agar and supplemented with various concentrations of 2,4-D (0.5-2mg/l) alone and IAA (0.5-2mg/l) in combination with 2,4-D (1mg/l). The pH of the medium was adjusted between 5.6-5.8 and autoclaved for 20 minutes at 121°C for 15 lbs pressure (Sahu, 2015; Reddy, 2014).

Culture condition:

The growth condition was maintained at 25 ± 2°C and the light intensity was 3000 lux with a photoperiod of 18 hrs daylight and 6 hours dark. The cultures were subcultured every 15 days with fresh MS medium.

Analysis of biomass:

After 30 days of culture, the callus sample was collected and blotted in tissue paper. Then, it was allowed to dry at room temperature and fresh weight (FW) was measured. The callus was dried at 50°C for 5 hrs in a hot air oven and the dry weight (DW) of the callus was calculated.

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Sample extraction procedure:
1g of the leaf and callus samples were powdered with liquid nitrogen. It was mixed with 10ml of methanol and kept in an orbital shaker at 150 rpm for 3 days. The samples were covered with aluminium foil in order to prevent photo-oxidation. After 3 days, the samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was taken and the content was transferred into an amber bottle and allowed for evaporation. Once the solvent is completely evaporated, the dried extract is weighed and stored at 4° C. For analysis, the prepared leaf and callus sample was prepared with 1 mg/ml concentration and dissolved in methanol (Parkhe et al, 2019).

Phytochemical screening:
Qualitative phytochemical screening:
For qualitative phytochemical screening, we followed the protocol from Nancy et al., 2014 and Parkhe et al, 2019 for checking the presence or absence of phenols, flavonoids, alkaloids, tannins, terpenoids, glycosides, saponins, and phytosterols.

Quantitative phytochemical screening of phenols:
The Folin-Ciocalteu Spectrophotometric method was used for the determination of total phenolic content. To the samples and standard solution (gallic acid), 125 µl of Folin-Ciocalteu phenol reagent was added to this mixture and shaken well. After 6 minutes, 1.25 ml of 7 % sodium carbonate solution was added to the mixture. A standard curve was developed using different concentrations of gallic acid (25µg/ml, 5µg/ml, 75µg/ml, and 100µg/ml). The reaction mixture was incubated for 90 minutes at room temperature. The absorbance values for test and standard solutions were noted against blank at 765 nm with a UV /Visible Spectrophotometer. The total phenol content was determined as mg GAE (gallic acid equivalent)/g of crude extract (El Hajaji 2010).

Quantitative phytochemical screening of flavonoid:
The aluminium chloride colorimetric assay was employed for quantifying flavonoids in the crude extracts. 1.5 ml of 95% ethanol was added to 0.5 ml standard solution (quercetin) and samples. To this, 0.1 ml of 10 % aluminium chloride was mixed. Thereafter, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water were added. The solution was kept for incubation at room temperature for 20 minutes. The absorbance readings were recorded for test and standard solutions against blank at 415 nm in a UV/Visible spectrophotometer. A standard curve was prepared with standard and sample values. The total flavonoid content was expressed as mg QE (quercetin equivalent)/g of crude extract (Nancy and Ashletha, 2015).

Antioxidant assays:
Determination of antioxidant activity using the DPPH radical scavenging method:
The free radical scavenging activity of the ethanolic extract was determined. Briefly, 0.1 ml of extract and standard compound ascorbic acid of variable concentration (20-100 g/ml) were taken in separate tubes. 450µl of Tris HCl and 1ml of 0.1mM DPPH (0.1mM/L in methanol) were added. The samples were incubated for 30 min in the dark and the absorbance of the reaction mixture was measured at 517 nm spectrophotometrically (Shimadzu UV-1609, Japan). The ascorbic acid was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent of inhibition activity was calculated as [(Ac-Ae)/Ac] 100, where Ac is the absorbance of the control and Ae is the absorbance of the extract/standard (El Hajaji 2010).

Determination of antioxidant activity using the ferric reducing/antioxidant method (FRAP):
The FRAP assay was done according to the method described by Benzie and Strain (1996) with some modifications. The stock solutions include 300 mM acetate buffer (3.1 g C2H3NaO2. 3H2O and 16 ml C2H4O2), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution. The ascorbic acid was used as standard. The fresh working solution (FRAP reagent) was prepared by mixing 50 ml acetate buffer, 5 ml TPTZ solution, and 5 ml $FeCl_3$. 6H2O solution (ratio 10:1:1). To 300 µl of callus and leaf extract samples, 2.7 ml of the FRAP reagent was added. After incubation for 5 minutes in room temperature, the absorbance was read at 593 nm. The difference in absorbance between the blank and each sample is compared to the total ferric-reducing antioxidant power (FRAP value) of the antioxidants in the sample.

Statistical analysis:
The data were statistically analyzed using analysis of variance using SPSS version 11.09 (IBM Corporation). Data are represented as mean ± standard error. The mean separations were carried out using Duncan's multiple range test (DMRT), and the significance was determined at 5% level.

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Results:

Molecular docking of selected *Vitex* secondary metabolites with HER2 target protein:

Molecular docking has been an efficient method for the identification and discovery of new molecular drug candidates. In this study, various potential HER2 inhibitory compounds were identified with best binding energy from leaf extracts of *Vitex negundo* (Table-1). In terms of free binding energy, these *in silico* findings revealed that D-Glucose, 6-O—D galactopyranosyl compound shows maximum effective binding energy against HER2 protein. These interactions with the ligand were favored by the residues: Thr94, Gln39, Gly70, Ile199, and Tyr217. In the event of identifying the lead molecule, D-Glucose, 6-O--D-galactopyranosyl was considered as a potential inhibitor of HER2 among other phytocompounds. In terms of free binding energy, these *in silico* findings revealed that D-Glucose, 6-O--D-galactopyranosyl- compound shows maximum effective binding energy against HER2. However, figure 1 shows the ligand interaction of D-Glucose, 6-O-D-galactopyranosyl-compound with HER2 protein.

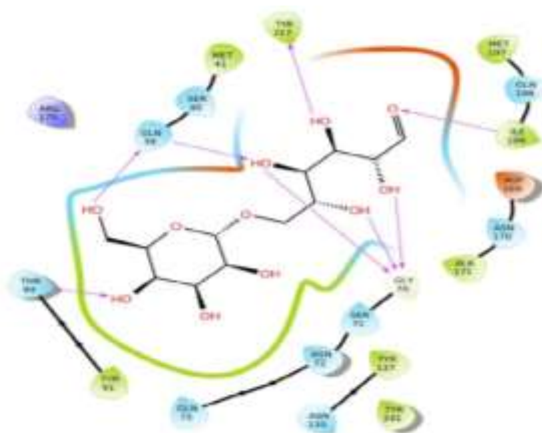

Fig.1. Molecular docking and ligand interaction of D-Glucose, 6-O-D-galactopyranosyl compound with HER2 protein

Table-1: List of *Vitex negundo* phytocompounds achieving best binding energy with the selected target HER2 protein

Compound name	Compound ID	Docking score
D-glucose, 6-O-D-galactopyranosyl-	44134813	-13.027
D-mannose	18950	-9.5
Benzoic acid, 3-hydroxy	7420	-5.658
12-bromo-13-hydroxy-2,5,9,13-tetramethyl tetradeca-4,8 dienoic acid,methyl ester	5367610	-5.54
4H-pyran-4-one, 2,3-dihydro -3,5-dihydroxy-6-methyl-	119838	-5.032
Aromadendrene oxide-1	528759	-4.945
2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-2-en-1-o	5369482	-4.772
ledol	92812	-4.46
caryophyllene	5281515	-4.432
2,3-dihydrothiophene 1,1 dioxide	14497	-4.322
2,4-pentadien-1-ol,3-propyl-2Z	5364724	-4.301
Azulene 1,4-dimethyl-7-(1-methylethyl)-	3515	-3.971
Hexanoic acid, ethyl ester	31265	-3.709

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Phytol	5280435	-3.534
Propane 1,1,3-triethoxy-	24624	-3.534
10,13-octadecadiynoic acid,methyl ester	28962	-2.113
Ethanol,2-(9-octadecenyloxy)-(Z)-	5364713	-1.937
Ethyl iso-allocholate	6452096	-1.804
Butane 1,1-diethoxy-3-methyl	19695	-1.776
6,9,12,15-Docosatetraenoic acid, methyl ester	5362672	-1.703
Ethanol,2-(9,12-octadecadienyloxy)-,(Z,Z)-	5365675	-1.45
4-Decynoic acid, methyl ester	549104	-0.917
4,9-Decadienoic acid, 2-nitro-, ethyl ester	5466561	-0.269
Hexadecanoic acid, ethyl ester	12366	0.922

***In vitro* callus induction:**

All the explants were able to produce white-colored callus on MS medium containing 0.5 -2.0 mg/l 2,4- D and in combination with IAA (0.5-2mg/l). Good callus response was observed when the concentrations of both auxins were high (Fig.2,3). The callus developed was friable, shiny and greenish cream colored. Among various concentrations, it was found that MS medium supplemented with 2,4-D (1.0 mg/l) in combination with IAA (0.5 mg/l) facilitated the highest percentage of callus response (81.2%), fresh weight (68.3±0.02mg) and dry weight (17.2±0.03mg), while other concentrations showed decreased response (Fig.4, 5).

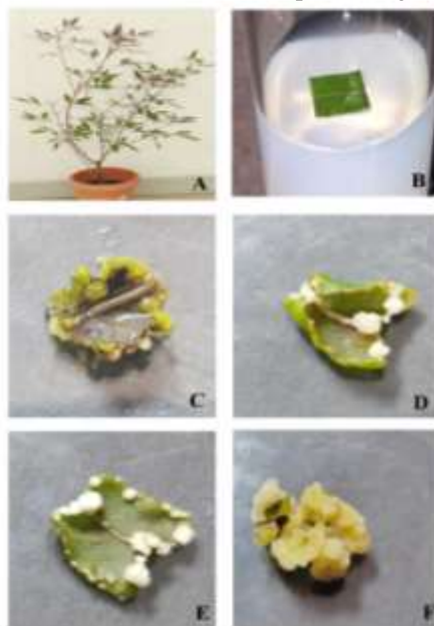

Fig.2: Callus induction and proliferation: (A) *Vitex negundo* grown in experimental garden of Bharathiar University; (B) Leaf explants (8 mm x 6 mm); (C) Callus induction after 5 days of culture; (D) Proliferation of callus after 10 days of culture; (E) Proliferation of callus after 20 days of culture; (F) Proliferation of callus after 30 days of culture. All the cultures were maintained on MS medium supplemented with 2,4-D (1mg/L) + IAA (0.5 mg/L). Subculturing was done every 15 days.

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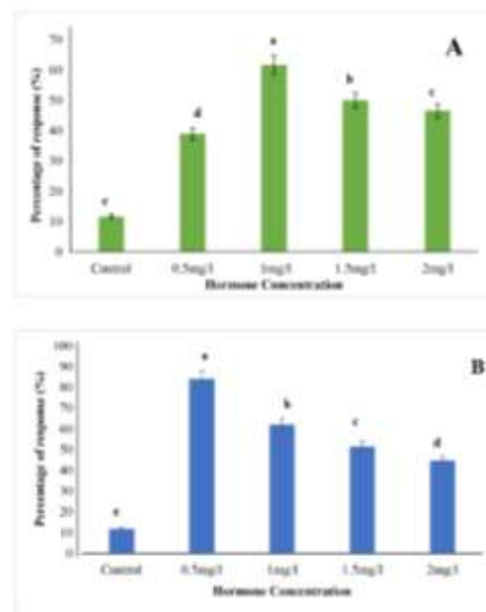

Fig.3: Callus induction from leaf explants of *Vitex negundo*: (A) Effect of 2,4-D (0.5 – 2mg/L); (B) Effect of IAA (0.5 – 2.0mg/L) in combination with 2,4-D (1mg/L). Percentage of response = (no. of explants responded / no. of explants inoculated) x100. The data were recorded after 30 days of culture. *Bars* represents the mean values of three independent experiments (n=10) with standard errors. Mean values followed by different letters are significantly different according to Duncan's multiple range test at 5% level.

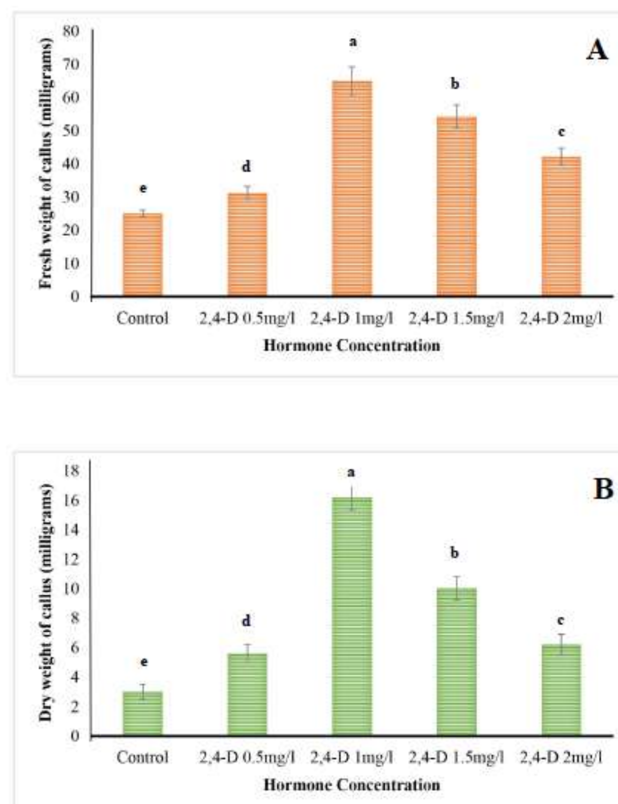

Fig.4: Effect of 2,4-D (0.5– 2.0mg/L) on biomass of callus induced from leaf explants of *Vitex negundo*: (A) Fresh weight/explant; (B) Dry weight/explant. The data were recorded after 30 days of culture. *Bars* represents the mean values of three independent experiments (n=10) with standard errors. Mean values followed by different letters are significantly different according to Duncan's multiple range test at 5% level.

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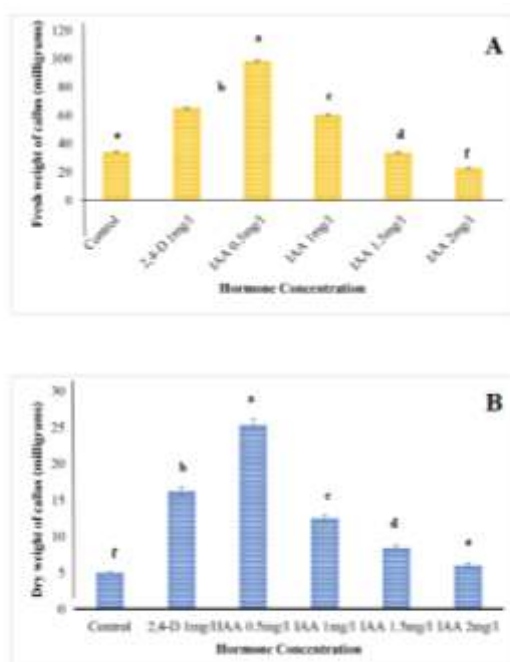

Fig.5: Effect of IAA (0.5 – 2.0 mg/L) in combination with 2,4-D best concentration (1mg/l) on callus biomass induced from leaf explants of *Vitex negundo*: (A) Fresh weight/explant; (B) Dry weight/explant. The data were recorded after 30 days of culture. *Bars* represents the mean values of three independent experiments (n=10) with standard errors. Mean values followed by different letters are significantly different according to Duncan's multiple range test at 5% level.

Qualitative phytochemical screening:

Phytochemical screening results show that leaves and callus extract gave a positive response to phenol, flavonoid, alkaloid, tannins, terpenoid, glycoside and, saponin and negative response to phytosterols (Table 2). The formation of deep blue or black color indicates the presence of phenolic compounds. The yellow color becomes colorless on the addition of dilute hydrochloric acid, indicating the presence of flavonoids. The presence of white creamy precipitate indicates the presence of alkaloids. The formation of blue or greenish color of the solution was observed. This was the indication of presence of tannins. The blue rings was observed instead of green rings which indicates the absence of terpenoids. The appearance of cherish red colour indicates the presence of glycosides. The persistence of foam was observed which indicates the presence of saponins. No colour change was observed in phytosterol test, depicting the absence of phytosterols in the samples.

Table 2: Qualitative phytochemical analysis of *V. negundo* methanolic extract

Phytochemical tests	Callus extract	Leaves extract
Phenol	+	+
Flavonoid	+	+
Alkaloid	+	+
Tannins	+	+
Terpenoids	+	+
Glycosides	+	+
Saponins	+	+
Phytosterols	-	-

"+" refers to the presence of phytocompounds; "-" refers to the absence of phytocompounds

Quantitative phytochemical screening of phenols and flavonoids:

In the present study, the concentration of total phenolic (40 ± 0.05 mg GAE/g of extract) and flavonoid content (25 ± 0.05 mg QE/g of extract) was high in leaf callus compared to control leaves (phenolic content = 38.8 ± 0.02 mg GAE/g of extract) and (flavonoid content = 22.5 ± 0.03 mg QE/g of extract) (Fig.6).

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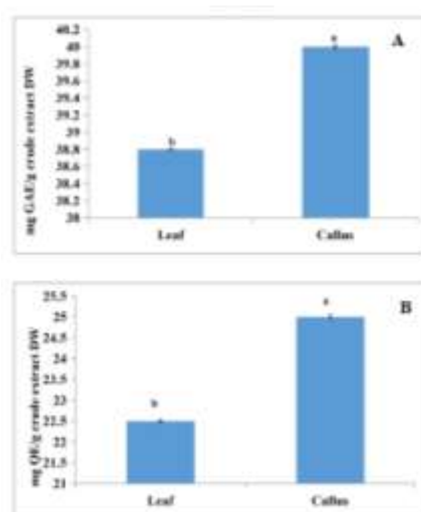

Fig.6: Comparative assessment of phytochemical assays in leaf-derived callus culture of *Vitex negundo* (A) Total polyphenolic content; (B) Total flavonoid content of leaf and callus. Bars represents the mean values of three independent experiments (n=5) with standard errors. Mean values followed by different letters are significantly different according to Duncan's multiple range test at 5% level.

Determination of antioxidant activity by DPPH radical scavenging method and ferric reducing/antioxidant method (FRAP):

In DPPH, the percentage of inhibition was higher in the callus (80.2%) when compared to control leaves (76.2%) and standard ascorbic acid (85.8%). Furthermore, the leaf extracts of callus were able to scavenge DPPH radicals in a concentration-dependent manner. The reducing ability of the control leaf and solid callus were determined using FRAP assay. The formation of blue-colored TPTZ-Fe2+ complex from colourless oxidized TPTZ-Fe3+ by the action of the electron donating antioxidants was recorded at absorbance 593 nm. In the FRAP assay, the callus sample (75.9%) showed elevated ferrous chelating activity when compared to control leaves (67.8%) and standard (90.3%) (Fig. 7).

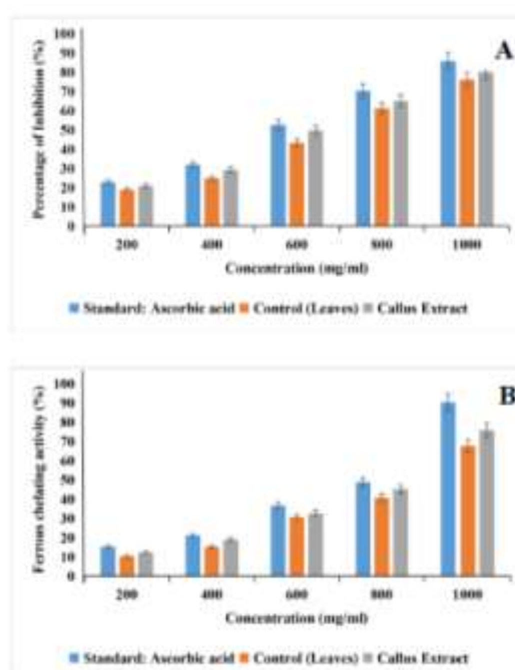

Fig.7: Comparative assessment of Antioxidant assays in leaf derived callus culture of *Vitex negundo* Control: Methanolic extract of *V.negundo* leaves from field grown plants, Standard: Ascorbic acid, Callus extract: Methanolic extract prepared using callus produced in MS media supplemented with 2,4-D 1 mg/l and

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IAA 0.5 mg/l. Bars represents the mean values of three independent experiments (n=5) with standard errors. Mean values followed by different letters are significantly different according to Duncan's multiple range test at 5% level.

Discussion:

Molecular docking simulation is one of the best significant methods to analyze the binding orientation of small complex molecules, drugs, and proteins (Tripathi, 2012; Mukherjee, 2021). The docking allows us to virtually screen the database of compounds and predict the strongest binders based on the various scoring functions. It is very helpful in studying the interactions of ligand molecules with the target protein before its *in vitro* synthesis. Among various medicinal plants, *Vitex negundo* is an important ethnobotanical plant and the phytochemicals present in the extracts have been reported to have various medicinal properties such as anti-inflammatory, anti-cancer, anti-diabetic, antioxidant, etc (Dharmasiri, 2003). Hence, the *Vitex* leaf-based phytocompounds were selected to carry out docking studies against the HER2 protein. The phytocompounds present in the leaf extract of *Vitex negundo* were screened for their binding affinity against HER2 through docking studies. The binding mode of the ligands within the active site of the HER2 protein was analyzed. The binding interaction between the ligand and residues Thr94, Gln39, Gly7, lle199 and Tyr217 was noted. It gives 2D visualization of the drug and receptor. Many secondary metabolites of *Vitex negundo* showed stronger binding affinity against HER2 which is highlighted in the table. In terms of free binding energy, these *in silico* findings revealed that D-Glucose, 6-O--D-galactopyranosyl- compound shows maximum effective binding energy against HER2. Therefore, this study can be a preliminary work for the development of new drugs against HER2-positive breast cancers (Zhou, 2009).

The plant cell culture techniques provide an appealing alternative for the production of valuable secondary metabolites, and they have been used throughout the years as a tool for the elucidation of secondary metabolite biosynthesis (Farjaminezhad, 2013). The diverse groups of compounds known as plant secondary metabolites include substances with pharmaceutical activity (e.g., morphine, vincristine), fragrances, pigments, latex, enzymes, and carbohydrates (Hunter & Kilby, 1990). It is possible to regenerate plants from variant cells, and selection techniques have the potential for the production of crop varieties with new characteristics such as herbicide resistance (Saunders *et al.,* 1992), salt tolerance cold tolerance, disease resistance and metal tolerance (Freytag, 1990). The effectiveness of callus induction depends on the medium formulation, type, and combination of growth regulators used. The characteristics of the callus (biomass, color, and texture) depend on the culture medium and the protocol of callus induction of *V. negundo* opens new vistas that could facilitate phytochemical production and extraction of pharmaceuticals from the callus without harvesting the plant itself (Chowdhury, 2011; Rao, 2002). Callus production was strongly influenced by the type and dose of auxin. Callus exhibited good growth within 30 days of inoculation. The callus from leaf explants were whitish green in colour and it proliferated from the midrib and further developed from the cut edges of the explants. A significant interaction between auxin type and dose was observed. The percentage of response in callus formation was found to be 80%. In addition to that, the media supplemented with 1mg/l 2,4-D and 0.5mg/l IAA was found to be most effective in promoting the callus production. In our experiment, auxin was proved to be necessary for high percentage for callus induction, 2,4-D alone and high concentration of 2,4-D and IAA would inhibit callus formation. Thus, an efficient protocol has been developed for induction and proliferation of callus in *Vitex negundo*.

On the other hand, qualitative and quantitative analysis along with antioxidant assays of such vital compounds is extremely significant to determine the quality and efficacy of plant secondary metabolites. The phenolic, and flavonoid compounds are important antioxidants that also include antimicrobial, antiallergic, anti-inflammatory, and anticancer agents. Among them, flavonoids have been used against cancer-causing tumors and it inhibits the promotion of growth and progression of tumors. Also, phenols when mixed with flavonoids in plants were reported to have multiple activities like antioxidant, anticarcinogenic, anti-inflammatory, etc (Agarwal, 2012; Durai, 2016; Singh, 2011). The methanolic extracts of *Vitex negundo* were also subjected to qualitative, quantitative and antioxidant studies. In the present study, qualitative phytochemical screening of methanol extract showed that the leaves and callus extract gave a positive response to alkaloids, saponin, terpenoid, phenol, flavonoid, tannins and a negative response to phytosterols (Table 2). In quantitative analysis, it was found that the concentration of total phenolic (40 ±0.05 mg/g) and flavonoid content (25±0.05mg/g) was high in leaf callus compared to control leaf (phenolic content = 38.8±0.02 mg/g); (flavonoid content = 22.5±0.03 mg/g). In the DPPH assay, the antioxidant potential was higher in the callus culture (80.2%) when compared to control leaves (76.2%) and standard (85.8%). In antioxidant assays, the reducing ability of the control leaf and callus was determined using FRAP assay. In the FRAP assay, the callus culture (75.9%) showed higher potency of inhibition when compared to control leaves (67.8%) and standard (90.3%) (Fig. 6). The chelating effects (%) of

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the methanolic extract and standard ascorbic acid on ferrous ion are presented in Figure 7. The percentage of metal chelating capacity of *Vitex negundo* extract, control with the standard was in the order of; ascorbic acid (90.3 %) > *Vitex negundo* callus extract (75.9%) followed by leaf extract (67.8%) at 1000 µg/ml concentration. Thus, the ability to chelate transition metals is considered to be an important antioxidant mode of action. It was reported that chelating agents, which form σ-bonds with a metal ion are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Farjaminezhad, 2013; Rani, 2006; Yahaya, 2018).

Conclusion:

In silico studies revealed the identification of HER2 inhibitory compounds present in the leaf extracts of *Vitex negundo*. The ligand interaction of D-Glucose, 6-O-D-galactopyranosyl- compound with HER2 protein showed the maximum binding affinity when compared with other compounds. However, these results are preliminary. In addition to that, a molecular docking study is only a way of predicting the activity of the molecules involved. In plant tissue culture, auxins proved to be necessary for the higher percentage of callus induction and proliferation. The highest callus biomass was achieved with 2,4-D (1mg/L) and IAA (0.5mg/L). It produced the highest percentage of callus response (81.2%) and callus biomass (fresh weight = 68.3 ± 0.02 mg; dry weight = 17.2 ± 0.03 mg). When compared to control leaves, the callus sample contained a higher amount of total polyphenolic (40 ± 0.05 mg GAE/g of extract), flavonoid (25 ± 0.05 mg QE/g of extract) contents and also exhibited an increased percentage of inhibition (80.2%), and ferrous chelating activity (75.9%). Therefore, further *in vitro* and *in vivo* studies need to be performed on animal models to confirm the anti-cancerous activity of *Vitex*-based compounds. From this study, it is summarized that the screened natural phytocompounds from *Vitex negundo* may serve as potential inhibitors for HER2 and they might lead to the development of new therapeutic targets against cancer.

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