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PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTICANCER ACTIVITY OF *TRIDAX PROCUMBENS LINN*

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ABSTRACT

Objective: To evaluate the phytochemical analysis and invitro anticancer activity of *Tridax procumbens* linn. To investigate the anticancer potential of ethanolic leaf extract of *Tridax procumbens* by 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, against MCF 7 (breast cancer cell line). **Materials and Methods:** The ethanolic extract of *T.procumbens* leaves was subjected to phytochemical analysis and antibacterial activity was done by disk diffusion method against few bacterial isolates followed by antioxidant studies. The docking studies were performed to check the anticancer potential by insilico method, finally the in vitro anticancer potential of *T.procumbens* was tested against MCF 7 cell lines through MTT assay. **Results:** The phytochemical constituents such as phenols, tannins, terpenoids, proteins, carbohydrates were present in our plant. The radical scavenging activity of the leaf extract was maximum at 250µl. The ethanolic leaf extract was found to be more effective against *Streptococcus sp* with the zone of inhibition of 22mm. The ethanolic extract of *T.procumbens* has the capability to reduce the size of the formazan crystals at the concentration of 100µg/ml. **Conclusion:** The present study concluded that the ethanolic leaf extract of *T.procumbens* has the anticancer potential against MCF cell lines. Further research is needed to isolate and characterize the specific anticancer compound from the plant extract and *in vivo* studies.

KEYWORDS: *T.procumbens*, phytochemical screening, antibacterial activity, antioxidant activity, insilico docking, anticancer activity.

INTRODUCTION

Cancer is a form of disorder characterized by enormous cell growth. There are more than a 100 types of cancer, and each is categorized by the type of cells that is predominantly affected. Cancer harms the body when transformed cells divide uncontrollably to form lumps or crowds of tissue called tumors (except in the case of leukemia where cancer excludes normal blood function by atypical cell division in the blood stream). Tumors grow and interfere with the digestive, nervous, and circulatory systems and they can discharge hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are considered to be benign.

Now a days, treatment for cancer can be done by chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy leads to a lot of strain and further damage in patient's body. Hence, people are moving to the alternative treatments and therapies against cancer.

Nature has been a wellspring of medicinal agents for thousands of years and an impressive number of current drugs have been isolated from inborn resources. Conventional medicine is a chief source of potentially useful new compounds for the advancement of chemotherapeutic agents **(Sunil Christudas *et al.*,).** *Tridax procumbens* from the family Asteraceae is often known as 'Ghamra' in local language and 'coat buttons' in English **(Vaishali Rai *et al.*,).** The wide range of curative applications is attributed to the existence of phytochemicals such as alkaloids, carotenoids, flavonoids, terpenoids, saponins and tannins. Hence it is worth withdrawing the phytoconstituents from *Tridax procumbens* and to examine their antioxidant activity **(vishnupriya *et al.*,)**. Tridax procumbens used either as an individual drug or conjugation with other drugs. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhea, high blood pressure and to verify hemorrhage from cuts, bruises, and wounds and to avert falling hair. The leaf extracts of this herb is also found to be exhibit antibacterial, antifungal, anticancer, anti -inflammatory and hepatoprotective. **(Sankaranarayanan1 *et al.*,).**

The aim of the current study was to estimate anticancer activity of ethanolic leaf extracts of *T. procumbens* on chosen cancerous cell lines i.e., MCF 7 by *in vitro* evaluation by 3-(4,5-dimethyl-thiazole2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

MATERIALS AND METHODS

Plant collection

Tridax procumbens leaves were collected from local area of Vayalur, Trichirappalli and the plant was authenticated by the Botany department of our college and the voucher specimen was obtained. The leaves chosen for the study had been washed, macerated and lyophilized. About 500g of the leaves yielded 33g of leaf powder. The technique was repeated to collect the required quantity.

Preparation of plant extracts

100g of the powdered peels were extracted in Soxhlet apparatus separately using 1 L of ethanol for 18h and then filtered. The filtrates were evaporated to dryness under reduced pressure and at a lower temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

Phytochemical screening

For qualitative phytochemical analysis, the ethanolic extract of leaves of *T.procumbens* plant were tested by using standard protocols.

Antibacterial activity

The antibacterial activity of the ethanolic leaf extract was tested against 5 bacterial isolates viz., *E.coli, Staphylococcus aureus Streptococcus sp, Klebsiella pneumoniae and Proteus sp.* All isolates were tested for susceptibility to the extracts and antimicrobial agents on Mueller Hinton agar by the standard disk diffusion method. The plates were then incubated overnight at 37℃.

Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) can be determined by adopting the procedure outlined by CLSI using the microtitre plates and were examined for bacterial growth (CLSI, 2006). The Minimal Inhibitory Concentration (MIC) assay is performed to determine the concentration of the extract that is lethal to the target bacteria *in vitro*.

DPPH radical scavenging assay

The ability of *T.procumbens* to scavenge 1, 1- diphenyl-2 picrylhydrazyl (DPPH) was measured by the reported method (Alothman *et al*., 2009). A mixture of absolute methanol and extract served as blank. Ascorbic acid was used as standard and different concentrations

of the extract (50,100,150,200 and 250 µl) were marked as tests. Finally DPPH reagent was added to all the test tubes including blank. Then, the absorbance of all samples was read at 515nm.

Calculation

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100.

Docking studies

PDB - The Protein Data Bank (PDB) is a source for the 3-D structural data of huge biological molecules, such as proteins and nucleic acids. The 3D structure of 1 mox protein from different mammals were retrieved using this.

Autodock - AutoDock is a type of automated docking tools. It is designed to calculate how small molecules, such as substrates or drug applicants, bind to a receptor of recognized 3D structure. AutoGrid calculates the energy of the noncovalent interactions between the protein and probe atoms that are situated in the different grid points of a lattice that defines the area of interest. The end result of these calculations the output file of the protein-ligand complex with stretchy residues and the ligand placed within the binding pocket is obtained. Each structure was scored and categorized by the program by the calculated communication energy.

Molecular docking study

MGL tools 1.5.4 with AutoGrid4 and AutoDock4 were used to accomplish docking calculations between the synthesized compounds and proteins. The crystal structure of beta-trypsin phosphonate inhibited was acquired from the protein data bank (http://www.rcsb.org./pdb). Receptor (protein) and ligand (synthesized compounds) files were prepared using AutoDock Tools.

At first, all the heteroatoms including water molecules were deleted. Lamarckian genetic algorithm, as implemented in AutoDock, was used to perform docking calculations. Further factors were set to default. The bottommost energy docked conformation, according to the AutoDock scoring task, was selected as the binding mode. The output from AutoDock was visualized using PyMol molecular graphics program.

Anticancer activity

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of viable cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader.

RESULTS AND DISCUSSION

Phytochemical screening

Secondary metabolites plays a vital role for the plant to rely on for medicinal property. Phytochemical studies were carried out for ethanolic leaf extract of *T.procumbens* to detect the presence of steroids, terpenoides, tannins, flavonoides, saponins, glycosides, amino acids etc. This study evidenced that the ethanolic leaf extract of T. procumbens contains the following secondary metabolites such as steroids, proteins amino acids, anthocyanins, phenols, flavonoids and terpenoids.[Table: 1]

Table 1: Phytochemical Screening – leaf Extract of *T.procumbens.*

S.No	Phytocompounds	Ethanolic extract
1.	Steroids	+
2.	Carbohydrates	+
3.	Proteins	+
4.	Aminoacids	+
5.	Anthocyanins	+
6.	Phenols	+
7.	Alkaloids	_
8.	Saponins	_
9.	Flavonoids	+
10	Terpenoids	+
11	Sugars	-

Antibacterial activity

The observation of the study reveals ethanolic extract as shown predominant inhibition against the bacterial pathogens. The potential of the plant extract was compared with the standard antibiotics. The result was recorded by measuring the zone of inhibition (ZOI). Among the different tested species, *Streptococcus Sp* was effectively inhibited by the plant extract. Followed by klebsiella.sp, *Staphylococcus.sp*, *Proteus.sp*, and *E.coli*. [Table: 2]. This

study reveals that the plant extract (50µl) was more effective when compared with the standard drugs [Table: 3] and can be used as a potent antimicrobial curative in future.

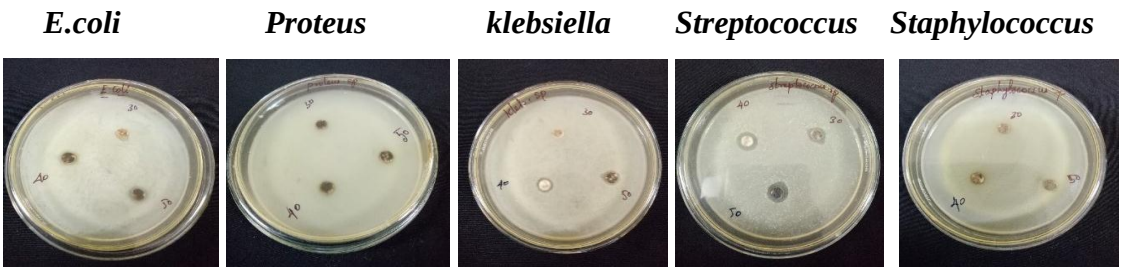


Fig 1: Antibacterial acticity of leaf extract.

Table 2: Antibacterial activity of ethanolic leaf extract.

S.No	Bacterial isolates	Plant extract (in mm)		
		30µl	40µl	50µl
1.	*E.coli*	4mm	6mm	12mm
2.	*Proteus sps*	2mm	4mm	8mm
3.	*Klebsiella sps*	5mm	9mm	11 mm
4.	*Streptococcus sps*	15mm	17mm	22mm
5.	*Staphylococcus sp*	-	-	-

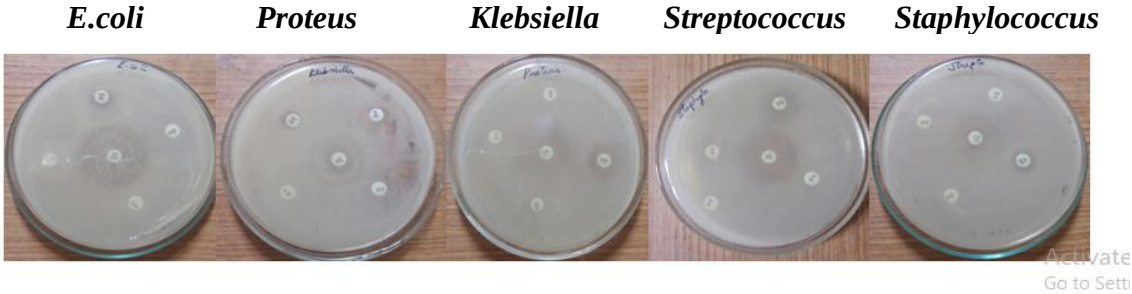


Fig 2: Antibacterial activity of standard antibiotics

Table 3: Antibacterial activity of standard antibiotics.

S.No	Bacterial isolates	Plant extract (in mm)		
		30µl	**40µl**	**50µl**
1.	*E.coli*	4mm	6mm	12mm
2.	*Proteus sps*	2mm	4mm	8mm
3.	*Klebsiella sps*	5mm	9mm	11 mm
4.	*Streptococcus sps*	15mm	17mm	22mm
5.	*Staphylococcus sp*	-	-	-

Minimal inhibitory concentration

The minimal inhibitory effect for the ethanolic extract of leaves of *Tridax procumbens* is found to be as effective dosage against the tested organism. The considered effect was found to be more in streptococcus.sp. [Fig: 3].

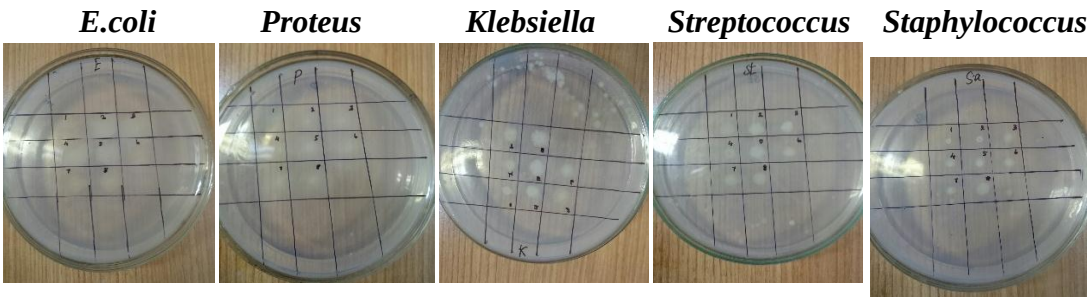


Fig 3: Minimal inhibitory concentration.

ANTIOXIDANT ACTIVITY

The DPPH radicals are widely used to investigate the scavenging activity. In the DPPH assay, the antioxidants has the capacity to reduce the stable radical DPPH to the yellow colored diphenyl- picrylhydrazine, resulting a colour change from purple to yellow. The absorbance declined when the DPPH was scavenged by an antioxidant through the contribution of hydrogen to form a stable DPPH molecule. [Table 4]

Table 4: Antioxidant activity of *T.procumbens.*

s.no	Concentration extract(µl)	OD value	% of antioxidant
1	50	3.00	21.2%
2	100	3.00	23.8%
3	150	0.492	48.96%
4	200	0.279	69.57%
5	250	0.057	98%

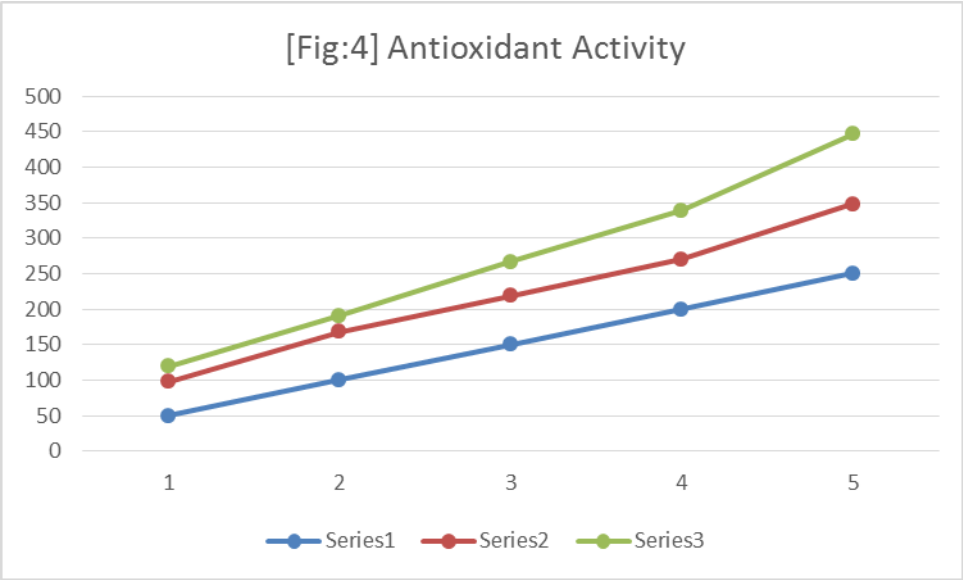


Fig 4: Antioxidant activity of *T.procumbens.*

Series 1- Concentration of plant extract

Series 2- Antioxidant % of ascorbic acid

Series 3- Antioxidant % of plant extract

In silico Docking study

Protein–small molecule docking investigations offer useful information to model the structure of biomolecule complexes and play an important role in drug development. To further authenticate the results of *in vitro* study, molecular docking stimulations were performed by docking the test compound with the protein I MOX (PDB ID: IQU3). Autodock and PyMol tools were employed in order to perform the docking studies. The docking poses of the compound with the target protein, and the obtained docking results were represented in [Table 5]. As shown in Table 5, the synthesized complex displays higher affinity for protein with the binding constant 8.03. This explains the higher anticancer potential of the present complex, which is in agreement with *in vitro* studies. It shows the phytocompounds of *T.procumbens* can fit well into the binding pockets (target protein 1MOX) and showing hydrophobic interactions with the amino acid residues like THR10, LUE38, GLY39, SER11, GLY10, HS409 [Fig:5]. It is clear from the literature survey that a minimum level of hydrophobicity favors the passage of drugs though cell membrane and their contact with intracellular targets.

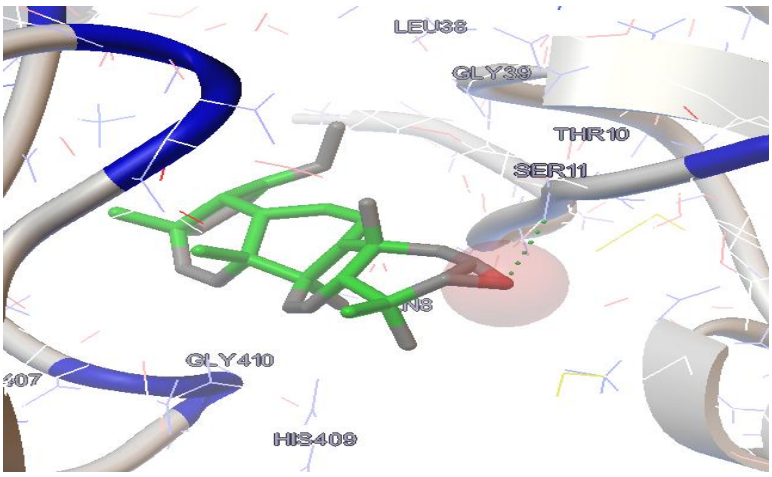


Fig 5: Surface representation of docked conformations of the present complex located within the hydrophobic pocket.

Table 5: Docking results of 1mox with selected compound.

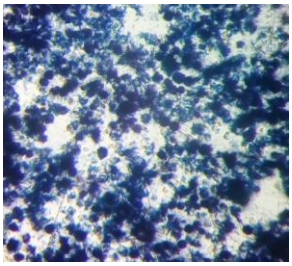
S.no	Protein	Compound	Compound id	Binding energy	Interacting residues
1	1 mox	Lupeol	259846	8.03	THR10,LUE38,GLY39, SER11,GLY10,HS409

Anticancer Activity

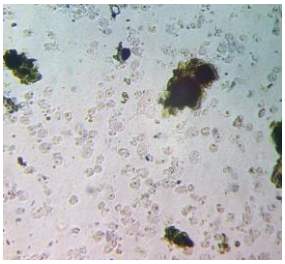
For the determination of anticancer potential the plant extract was taken and performed invitro studies against a MCF-7 cell line at different concentrations to determine the IC50 (56% growth inhibition) by MTT assay. MCF-7 cells were incubated with leaf extracts at 20 µg/ml, the amount of formazan was similar to control cells, while in other cells it was distinctly lower. The quantity of formazan produced varied between different concentrations of leaf extract treated cells. The formation of formazan crystals decreases when the concentration of a leaf extract increases for about 100µg/ml in MCF-7 cells. Though this study concluded that the ethanolic leaf extract of *T.procumbens* may be a potential drug for breast cancer in future. [fig: 6]

Table 6: OD Value of different concentrations of plant extract at 570 nm (control).

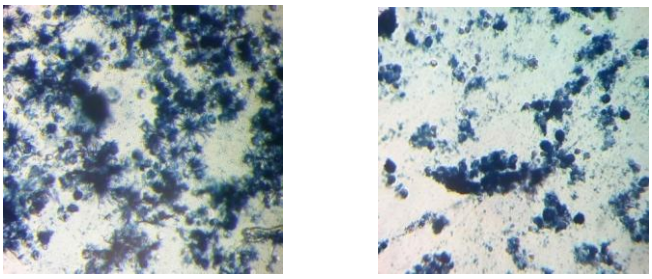
S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm (in triplicates)		
1.	Control	0.469	0.469	0.434
2.	100 µg/ml	0.155	0.136	0.110
3.	90 µg/ml	0.202	0.234	0.248
4.	80 µg/ml	0.245	0.269	0.234
5.	70 µg/ml	0.281	0.286	0.279
6.	60 µg/ml	0.307	0.301	0.250
7.	50 µg/ml	0.320	0.342	0.348
8.	40 µg/ml	0.349	0.311	0.377
9.	30 µg/ml	0.395	0.402	0.429
10.	20 µg/ml	0.404	0.430	0.406
11.	10 µg/ml	0.510	0.461	0.420



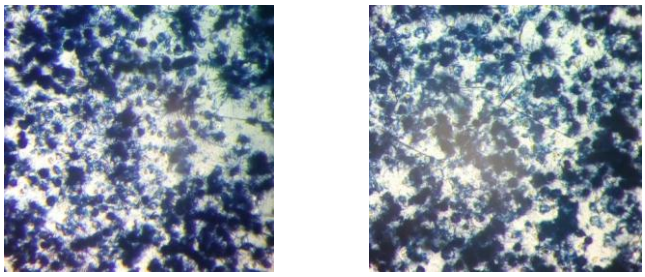
Control cells



Sample100 µg/ml



Sample 80µg/ml **Sample 60 µg/ml**



Sample 40 µg/ml **Sample 20 µg/ml**

Fig 10: Formation of formazan crystals in control cells and herbal extract treated cells.

CONCLUSION

The ethanol extract of *Tridax procumbens* showed the significant activity against bacterial pathogens such as *E.coli, K.pneumoniae, Proteus sp, Staphylococcus, Streptococcus sp,*. On phytochemical screening the ethanolic extract as revealed that the presence of biologically active important phytocomponents like as phenols, flavonoids, terpenoids, protein, Aminoacids, etc. In this case of antioxidant activity, DPPH is a stable nitrogen-centered free radical, the color of the extract which changes from purple to yellow upon reduction of free radical. It is found that the radical-scavenging activity of ethanolic extract of *T.procumbens* increased with increasing concentration. For the primary screening of anticancer potential insilico studies were carried out by Autodock tool. It shows the phytocompounds of *T.procumbens* can fit well into the binding pockets(target protein 1MOX) and showing hydrophobic interactions with the amino acid residues like THR10, LUE38, GLY39, SER11, GLY10, HS409.For the determination of anticancer potential the plant extract was taken and performed invitro studies against a MCF-7 cell line at different concentrations to determine the IC50 (56% growth inhibition) by MTT assay. MCF-7 cells were incubated with leaf extracts at 20 µg/ml, the amount of formazan was similar to control cells, while in other cells it was distinctly lower. The quantity of formazan produced varied between different concentrations of leaf extract treated cells. The formation of formazan crystals decreases

when the concentration of a leaf extract increases in MCF-7 cells. Though this study concluded that *T.procumbens* may be a potential drug for breast cancer in future.

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REFERENCES

1. Sathya bama S, Jayasurya kingsley S, Sankaranarayanan S and Bama P. Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens linn*.: identification and mode of action of the terpenoid compound as antibacterial. *International J of Pharma and Pharmaceut Sci*, 2012; 4(Suppl 1): 557-564.
2. Priya VP, Radhika K, Rao SA. In vitro anti-cancer activity of aqueous and acetone extracts of *Tridax procumbens* leaf on PC 3 cell lines. *Int J Pharm Pharm Sci*, 2011; 3(4): 1-4.
3. Vishnu Priya P, Radhika K, Sivakumar R, Boje Gowda B, Syed Sultan B, Prameela Devi Y, et al. In vitro anticancer activity of aqueous and acetone extracts of *Tridax procumbens* leaf on PC3 cell lines. *Int J Pharm Pharm Sci*, 2011; 3: 1-4.
4. Bhagwat DA, Killedar SG, Adnaik RS. Antidiabetic activity of leaf extract of *Tridax procumbens*. *Int J Green Pharma*, 2008; 2(2): 126-8.
5. Farhadi, K.; Esmaeilzadeh F.; Hatam, M.; Forough; M. & Molaie,R, *Food Chemistry*, 2016; 199: 847–855.
6. Diwan, P.V., Tilloo, L. D., Kulkarni, D. R.: Influence of T. procumbens on wound healing. Indian]. *Physiol. Pharmac*, 1982; 27: 32-36.
7. Ferguson, R.W. & Weissfeld, A.S. Comparison of the suitability of three common bacterial media for susceptibility testing oftrimethoprim-sulfamethoxazole. Clin. Microbiol, 1984; 19: 85–86.
8. Satya BS, Jayasurya KS, Sankaranyanan S, Bama P. Antibacterial Activity of Different Phytochemical Extracts from the Leaves of T. Procumbens Linn.: Identification and Mode of Action of the Terpenoid Compound as Antibacterial *International Journal of Pharmacy and Pharmaceutical Sciences*, 2012; 4: 557564.
9. Njoku OV, Obi C. (Phytochemical constituents of some selected medicinal Plants). *African Journal of Pure and Applied Chemistry*, 2009; 3(11): 228-233.

10. Satya BS, Jayasurya KS, Sankaranyanan S, Bama P. Antibacterial Activity of Different Phytochemical Extracts from the Leaves of T. Procumbens Linn.: Identification and Mode of Action of the Terpenoid Compound as Antibacterial International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4: 557564.
11. Bauer AW, Kirby WMM, Sherries JC, Turckp M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am J Clin Pathol, 1966; 45: 493496. 17.
12. Y.Z. Fang and R.L. Zheng, Biological Theory and Application of Free Radical, Science Press, Beijing, 2002.
13. Taddei A, Rosas-Romero AJ. Bioactivity studies of extracts from Tridax procumbens. Phytomedicine, 2000; 7: 235-238.
14. Mohamed Sham Shihabudeen H, Hansi Priscilla D, Kavitha T. Antimicrobial activity and phytochemical analysis of selected Indian fol.
15. M Kagoshima; T Wilcke; K Ito; L Tsaprouni; PJ Barnes; N Punchard. Eur J Pharmacol, 2001; 429: 327–34.
16. Saxena VK, Albert S. β-Sitosterol-3-O-β-Dxylopyranoside from the flowers of *Tridax procumbens Linn. J Chem Sci*, 2005; 117(3): 263-6.
17. Sawanth RS, Godghate AG. Preliminary phytochemical analysis of leaves of *Tridax procumbens Linn. Int J Sci Environ Technol*, 2013; 3: 388–94.
18. Agarwal S, Khadese S, Talele G. Bioactive immunomodulatory fraction from Tridax procumbens. Science Alert, 2010; 3: 120-127.
19. Elenbaas, B. et al. Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. Genes Dev, 2001; 15: 50–65.
20. Zhang, X. et al. Human growth hormone-regulated HOXA1 is a human mammary epithelial oncogene. J. Biol. Chem, 2003; 278: 7580–7590.