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# ANTIOXIDANT AND ANTILEUKEMIC ACTIVITY OF *VITEX NEGUNDO* LEAF EXTRACTS

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**Keywords:**

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**ABSTRACT:** *Vitex negundo* is an Indian medicinal plant used to cure several diseases such as asthama, fever, headache, cancer and many other diseases. This study aims to determine the antioxidant potential and anti-proliferative activity of *Vitex negundo* methanolic leaf extract. Isolation of bioactive compounds involved liquid-liquid extraction of methanolic leaf extract by petroleum ether and chloroform. The total antioxidant potential of the extracts was determined using phosphomolybdenum, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric oxide reducing power (FRAP) and Nitric Oxide methods. It was observed that the crude and chloroform extracts had good antioxidant activity. The antileukemic activity of the chloroform extract was tested on HEL92.1.7 and Jurkat cell lines (Acute lymphoblastic leukemia derived) by cytotoxicity assay (MTT). The plant extracts exhibited poor cytotoxicity against the erythroid cell lines but showed good cytoxicity on Acute lymphoblastic leukemia cells. This study shows that the chloroform fraction of *V negundo* leaf has both antioxidant and antileukemic activity.

**INTRODUCTION:** In the 20th century, cancer is the second cause of mortality all over the world of which Leukemia contributes to 53% of all cancers [1]. Acute lymphoblastic Leukemia (ALL) is an acute form of leukemia which is prevalent in children. Acute Erythroid leukemia (AEL) is a rare form of Acute myeloid leukemia (AML) which mainly affects adult population. It accounts for 5% of AML [2] with proliferation of abnormal erythroids, myeloblasts and megakaryocytes. Since chemotherapy treatment with allopathic drugs causes severe side effects there is a need for natural products which will be cost effective and have less side effects.

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Herbal and natural products have been used as folk medicines since centuries. Presence of various secondary metabolites in plants has paved way for isolation of compounds of pharmaceutical importance [3]. Various parts of the plants such as roots, barks, stem, leaves, fruits and seeds have been used to treat various ailments.

Oxidative stress due to life style and pathological conditions is associated with various diseases like cardiovascular, cancer and other chronic diseases. Oxidative stress generates lot of reactive oxygen species which includes free radicals such as super oxide, hydrogen peroxide and nitric oxide [4]. Isolation of antioxidant and anticancer phytochemicals to treat several ROS related diseases such as cancer and other diseases is increased in recent years and this in turn has lead to the discovery of new pharmaco-therapeutic agents [5, 6]. *Vitex negundo*, also known as the Chinese Chaste tree is classified under the kingdom *Plantae*

and is a member of the *Verbanaceae* family. This family consists of 250 species in which most of them have medicinal value [7]. It is a woody, aromatic shrub commonly found throughout the Indian subcontinent on riverbanks, moist localities and deciduous forests. The shrub grows to 2- 4m in height. *Vitex negundo* has been used to cure several ailments such as asthama, cancer, fever, head ache, wounds, antidote for snake bite, Rheumatism etc., [8] *Vitex negundo* leaf extract is known to be rich in iridoid glycosides, isomeric flavones, flavonoids and terpenoids [9]. Therefore the total phenolic content and flavonoids of this plant extract is a good measure of its antioxidant and anti-tumour effects [10]. *Vitex negundo* is also a source of natural antioxidants [11]. Recently studies have proved that the ethanolic extract of *Vitex negundo* increased the activities of antioxidant enzymes in normal embryonic liver cells (WRL68) including, superoxide dismutase (SOD) and glutathione peroxidase (GPX) [12].

Folk medicine from Andhra Pradesh and Phillipines suggest the use of *Vitex negundo* against cancer. Ethanolic extract of *Vitex negundo* has increased the life span of mice having EAC tumours by reducing the number of tumour cells. Recent studies have also shown that the extract can be used as chemopreventive agent [13]. Lignan compound EVn-50 isolated from *Vitex negundo* has broad spectrum cytotoxicity by arresting cancer cell lines at G2/M phase cell cycle [14]. The present study is an attempt to determine the antioxidant and antileukemic effect of *Vitex negundo* leaf extract.

## MATERIALS AND METHOD:

### Plant material:

Plant collection was done during the winter months of January- February, 2013 from, Atibele, Annekal, Karnataka. Authentication was done by Prof. Srinath, Department of Botany, Bangalore University. The leaves were washed under running water and dried with starch-free muslin cloth. The uninfected leaves were spread on a clean sheet and shade dried for 10 days. After shade drying the leaves were dried in hot air oven for 10 hours at 35°C. Dried leaves were coarsely powdered using conventional mixer(650W).

### Preparation of Extracts:

160g of the leaf powder was extracted using 1.5lt of 100% (v/v) methanol. The obtained extract was concentrated using rotary evaporator. The concentrated extract was further evaporated to a semisolid mass. The crude extract was fractionated using petroleum ether and chloroform. The concentrated petroleum ether and chloroform fraction were further evaporated to dryness or semisolid mass using rotary evaporator. The concentrated extract was stored at $4^0$C for further studies [15, 16].

### Total phenolic content:

Total phenolic content of the extracts (crude, petroleum ether and chloroform) was estimated by Folin ciocalteu method [17]. 100 µl of diluted plant extract and different concentrations of the standard (20 - 100µg) were taken in different test tubes. Total volume was made upto 1ml with methanol. 2.5ml of FCR reagent was added and were incubated at room temperature for 5 minutes. 2ml of sodium carbonate and 5ml of distilled water was added to each test tube. After 15minutes of incubation OD was read at 670nm using UV spectrophotometer. Gallic acid was used as standard. Methanol was used as blank.

### Antioxidant activity determination of *Vitex negundo* extracts:

### Determination of total antioxidant capacity by phosphomolybdenum method:

The total antioxidants in the extracts (crude, petroleum ether and chloroform) fractions were determined by Phosphomolybdenum method [18]. Diluted plant extracts and different concentrations of standard (10 – 50 µg) were taken in different test tubes. Final volume was made upto 0.5ml with distilled water. 2.5ml of the reaction mixture which contained 0.6M sulphuric Acid, 28mM sodium phosphate, 4mM ammonium molybdate was added and incubated at 95ºC for 60 minutes. 5ml of distilled water was added to all the test tubes after cooling and absorbance was read at 670nm using UV spectrophotometer. Ascorbic acid was used as standard. Distilled water was used as blank. Antioxidant capacity was expressed in µg AAE/ml.

**Ferric reducing antioxidant power (FRAP) assay:** The total antioxidant capacity of the extracts was measured by FRAP assay [19]. 100 µl of diluted

plant extracts and between 20 – 100 µg of different concentrations of the standard were taken in separate test tubes. 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferric cyanide was added and incubated at 50ºC for 20min. The reaction was stopped by adding 2.5ml of 1% trichloroacetic acid and centrifuged at 3000rpm for 10minutes. To 2.5ml of the supernatant, 2.5ml of distilled water and 1ml of 0.1% $FeCl_3$ was added and incubated at room temperature for 30 minutes. Absorbance was read at 700nm. Gallic acid was used as standard. Distilled water along with other ingredients was used as blank. Antioxidant capacity of the extract were expressed in terms of µg/ml Gallic acid equivalence per ml.

**Nitric oxide assay:**

The scavenging activity of the extracts (Crude, petroleum ether and chloroform) was measured by Nitric Oxide assay [20]. 100 µl of diluted plant extracts and 20 – 100 µg of different concentrations of the standard were taken in separate test tubes. 2ml of 10mM Sodium nitroprusside and 0.5ml of Phosphate Buffer Saline was added to the test tubes and incubated for 2hrs and 15 minutes at room temperature. 1ml of sulphanilic reagent (0.33% of Sulphanilic Acid is prepared using 20% Glacial Acetic Acid Reagent) was added and incubated for 5minutes at room temperature followed by the addition of 0.1% of Naphthylene Diamine Dihydrochloride (NEDD) reagent. It was further incubated for 15 minutes at room temperature. Ascorbic acid was used as standard. Distilled water was used as blank. Absorbance was read at 540nm. Percentage scavenging activity was calculated using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{(Absorbance of the control} - \text{Absorbance of the sample)}}{\text{(Absorbance of the control)}} \times 100$$

$IC_{50}$ was calculated using standard graph.

**DPPH assay:**

Free radical scavenging capacity was measured by DPPH method [21]. Various concentrations of the standard (100 -500 µg) and 100 µl of diluted extracts were taken in test tubes. Volume was made upto 150 µl and equal volumes of DPPH solution was added. It was incubated at room temperature for 15 minutes in a rotary shaker followed by the addition of 3ml methanol. Absorbance was read at 570nm. Ascorbic acid was used as standard. Methanol and DPPH mixture was used as control. Methanol alone was used as blank. Percentage scavenging activity was calculated using formula:

$$\text{Scavenging activity (\%)} = \frac{\text{(Absorbance of the control} - \text{Absorbance of the sample)}}{\text{(Absorbance of the control)}} \times 100$$

$IC_{50}$ was calculated using standard graph.

**Cytotoxicity assay:**

HEL.92.1.7 (erythroid leukemia cell line) and Jurkat cells (Acute lymphoblastic Leukemia cell line) were cultured in ATCC formulated RPMI 1640 medium. Cell lines were procured from ATCC. All cell lines were incubated in $CO_2$ incubator having 5% $CO_2$ and subcultured as needed (ATCC Guide). The viability of cells were assessed using MTT assay [22]. 200 µl (1 X $10^5$ cells/well) were seeded to each well of 96 well microtitre plate and incubated for 24hrs at 37ºC, 5 % $CO_2$ incubator. Different two fold varied concentrations of the chloroform extract were incubated ranging from 0-320 µg/ml (0, 5, 10, 20, 40, 80, 160 and 320 µg/ml) for 24hrs at same conditions. 96 well plate was centrifuged at 2000rpm for 5mins after incubation and supernatant was discarded carefully. MTT dye was added to the well and further incubated for 3 - 4hrs. After incubation MTT reagent was discarded without disturbing cells and DMSO was added quickly to solubilise formazan. Absorbance was measured at 590nm. The wells containing 1% DMSO was treated as blank. The 50% inhibition concentration ($IC_{50}$) of the extract was determined using graph pad prism software. Inhibition of cell growth was determined by the ability of the remaining viable cells to convert yellow dye MTT to purple coloured formazan. The cytotoxic ability caused by the plant extracts at the lowest concentration by 50% in treated compared to untreated culture was compared for their activities.

**RESULTS AND DISCUSSION:**

**Total phenolic content estimation:** The crude, chloroform and petroleum ether fractions were tested for total phenolic content and results are as shown in the **Fig. 1**. The total phenolic content in the crude extract and chloroform fraction was found to be 257.33mg/g GAE and 293.38mg/g GAE respectively. Total phenolic content was

higher in chloroform fraction. Earlier studies [23] have shown that the total phenolic content in the leaf extract was 249.96 ± 8.34 mg/g GAE of extract which correlated with our observation. Recent studies [24] have shown that the crude and chloroform extract had 90.1 and 56.3 GAE/g respectively. The present study shows higher total phenolic content in both fractions compared to earlier studies [24].

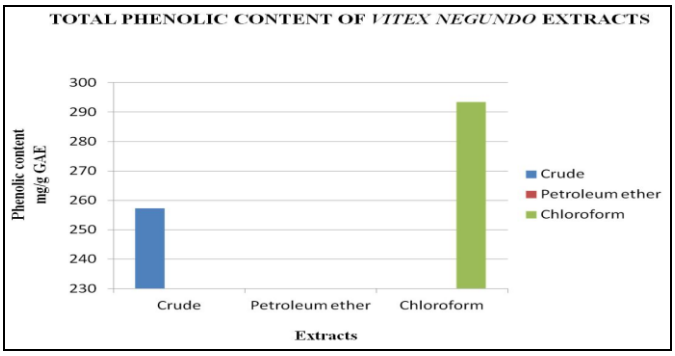



**FIG. 1: ESTIMATION OF TOTAL PHENOLIC CONTENT BY FOLIN CIOCALTEU METHOD**

## DPPH assay:

The scavenging activity for crude, chloroform and petroleum ether fractions was estimated by DPPH assay. The results are shown in the **Fig. 2**. The $IC_{50}$ value was high in chloroform fraction (330µg/ml) compared to the crude fraction. The above result clearly states that the crude extract has good scavenging activity compared to other extracts. Earlier studies have reported that the DPPH inhibitory activity of *Vitex negundo* was 69.82 ±2.99% at a concentration of 50mg/ml [23]. This value is in agreement with the results obtained from this study. Shah *et al* have reported $IC_{50}$ values of methanolic crude extract and chloroform extract as 23.4 and 51.3µg/ml respectively which further substantiates our results [24]. Petroleum ether fraction did not have scavenging ability.

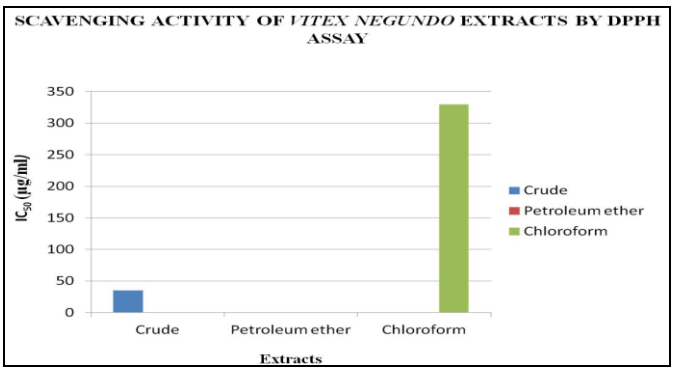



**FIG. 2: DPPH ASSAY**

## Determination of Antioxidant potential by Phosphomolybdenum method:

The total antioxidant capacity was estimated by phosphomolybdenum method. The results are shown in **Fig.3**. Crude and chloroform extract have shown very good antioxidant potential. The chloroform fraction showed greater than 50% activity indicating that it is a potent antioxidant [25]. The petroleum ether fraction had activity that was slightly greater than 50% and had a value of 56.67%. Studies [11] have reported that the methanolic crude extract had 341.66±13.60 mg/AAE g dry wt. The present study showed that the crude extract had higher antioxidant potential when compared to their study.

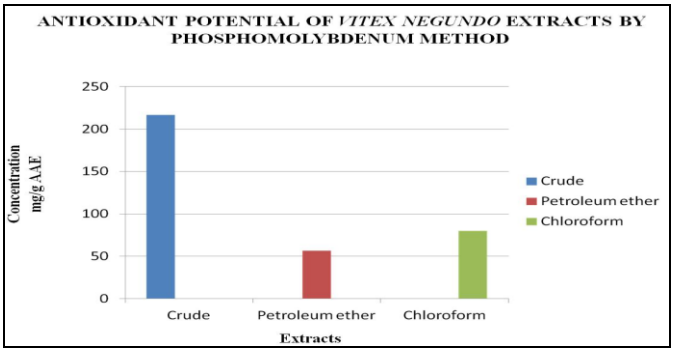



**FIG. 3: TOTAL ANTIOXIDANT POTENTIAL ESTIMATION BY PHOSPHOMOLYBDENUM METHOD**

## Nitric oxide assay:

The ability of *Vitex nigundo* to scavenge fifty percentage of nitric oxide free radicals was high in crude and chloroform extract which was 25.06 and 25.07µg AAE/ml respectively (**Fig.4**). Murali Krishna *et al* [26] reported that the stem methanolic extract exhibited a very high scavenging activity when compared to petroleum ether fractions (52.26µg AAE/ml). However, in the present study chloroform fraction of leaf extract has shown very good radical scavenging activity.

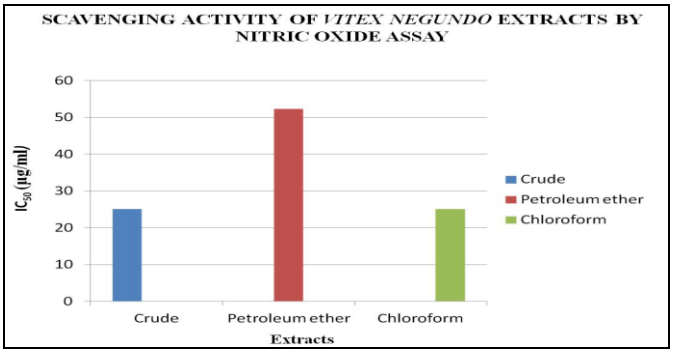



**FIG. 4: ESTIMATION OF SCAVENGING ACTIVITY NITRIC OXIDE ASSAY**

**FRAP assay:**

The antioxidant potential of crude and chloroform fraction extract was found to have 100 and 57µg GAE/ml respectively (**Fig. 5**). Crude fraction is showing high antioxidant potential. The present result is in line with the results reported by [27]. According to their study methanolic crude extract had 44.6 ± 7.8 µM TE/g. In the present study crude methnolic extract as well as chloroform fraction have good antioxidant potential. Ethanolic crude extract had 866.11 µmol Fe (II)/g reducing ability [12] from previous studies.

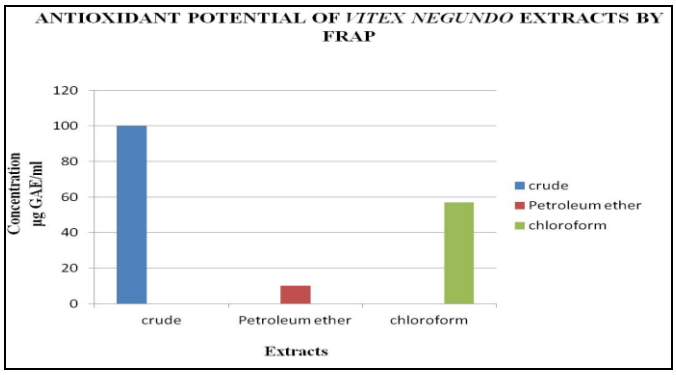



**FIG.5: DETERMINATION OF ANTIOXIDANT POTENTIAL BY FRAP ASSAY**

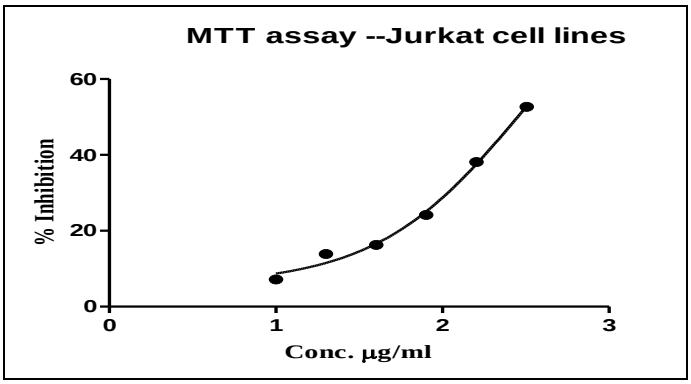
The present study reports that the methanolic and the chloroform leaf extract are a good source of antioxidants. Petroleum ether fraction has not shown good antioxidant activity except in nitric oxide and phosphomolybdenum assay.

## Cytotoxicity results

**TABLE 1: PERCENTAGE INHIBITION OF *VITEX NEGUNDO* CHLOROFORM LEAF EXTRACT ON JURKAT CELLS**

| Concentration (µg /ml) | % Inhibition | $IC_{50}$ µg/ml |
|---|---|---|
| 1%DMSO | 0.00 | |
| 10 | 7.20 | |
| 20 | 13.87 | |
| 40 | 16.25 | 291.5 µg/ml |
| 80 | 24.18 | |
| 160 | 38.14 | |
| 320 | 52.65 | |

*In vitro* cytotoxicity assay (MTT assay) of chloroform extracts of *Vitex negundo* against Jurkat cell line in a dose dependent manner is shown in graph 1 and 50 percent inhibition ($IC_{50}$) was 291.5 µg/ml (**Table 1).** The same chloroform fraction had less cytotoxic effect against HEL.92.1.7 (data not shown). Methanolic crude extract had maximum cytotoxicity (48.14±0.92 %) against THP-1 leukemia cell lines according to the studies of [11] . In the present study chloroform fraction which had good antioxidant activity was not effective on HEL.92.1.7 but was effective against jurkat cells (ALL derived). Studies done by [11] used crude extract against acute monocytic leukemia cell lines. Recently, studies on antiproliferative effect of methanolic leaf extracts of *Vitex negundo* on seven different cell lines (but not leukemia) [28] had very good antiproliferative effect on MDA-MB-231 with $IC_{50}$ values 65.38 µg/mL. The present study suggests that the chloroform fraction has antileukemic activity.




**GRAPH 1: $IC_{50}$ VALUE OF *VITEX NEGUNDO* CHLOROFORM LEAF EXTRACT ON JURKAT CELLS**

Our preliminary studies have shown that chloroform fraction of *Vitex negundo* leaf extract has good cytotoxic effect on acute lymphoblastic leukemia cell lines. Further purification of the chloroform fraction might result in a single compound with a good antiproliferative effect.

**CONCLUSION:** The results of free radical scavenging, antioxidant potential and antileukemic activity have shown that the chloroform fraction of *Vitex negundo* leaf extract had good antioxidant and antiproliferative activity. Although the crude extract had good antioxidant potential, chloroform fraction have shown better antioxidant potential and anti ALL effect. Therefore chloroform fraction was used to test on cell lines. Since the chloroform fraction had antiproliferative activity on ALL cell lines it could have potent anti ALL compounds. Further purification of the chloroform fraction could lead to the identification of a potent drug candidate for treatment of ALL.

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**CONFLICT OF INTEREST:** All authors declare no conflict of interest.

## REFERENCES:

1. Chueahongthong F, Ampasavate C, Okonogi S, Tima S and Anuchapreeda S. Cytotoxic effects of crude kaffir lime (*Citrus hystrix* DC) leaf fractional extracts on leukemic cell lines. J Med Plants Res 2011; 5(14): 3097-3105.
2. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Beau L M., Hellström-Lindberg E, Tefferi A, and Bloomfield CD. The 2008 revision of World Health Organisation (WHO) classification of Myeloid neoplasms and Acute leukemia: rationale and important changes. Blood 2009; 114(5).
3. Khalafalla MM, Abdellatef E, Daffalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, Cocchetto A and El-Shemy HA. Antileukemia activity from root cultures of *Vernonia amygdalina*. J Med Plants Res 2009; 3 (8): 556-562.
4. Arokiyaraj S, Martin S, Perinbam K, Arokiyanathan P M and Beatrice V. Free radical scavenging activity and HPTLC finger print of *Pterocarpus sentalinus* L an *invitro* study. Ind J Sci Tech 2008; 1(7): 1-3.
5. M. E. El-Sabagh, K. S. Ramadan, I. M. A. El-slam and A. M. Ibrahim. Antioxidants Status in Acute Lymphoblastic Leukemic Patients. A J Med and Med Sci. 2011; 1(1): 1-6.
6. Manoj kumar, Amit kumar, Sukumar dandapat and Sinha MP. Phytochemical screening and antioxidant potency of *Adhatoda vasica* and *Vitex negundo*. The Bioscan 2013; 8(2): 727-730 (Supplement on Medicinal Plants).
7. Meena AK, Uttam Singh, Yadav AK, Singh B and Rao MM. Pharmacological and Phytochemical Evidences for the Extracts from Plants of the Genus *Vitex* – A Review. Int J Pharm Clin Res 2010; 2(1): 01-09.
8. Vishwanathan & Basavaraju. A review on *Vitex negundo* L. – A Medicinally Important Plant E J Bio Sci 2010; 3(1): 30-42.
9. Gautam LN, Shrestha SL, Wagle P and Tamrakar BM. Chemical constituents from *Vitex negundo* (Linn) of Nepalese origin. Scientific World. 2008; 6 No.6:27-32.
10. Preethi, R, Devanathan VV and Loganathan M. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Advan. Biol Res. 2010; 4(2): 122-125.
11. Thombre R, Jagtap R, and Patil N. Evaluation of phytoconstituents, antibacterial, antioxidant and cytotoxic activity of *Vitex negundo* l. and *Tabernaemontana divaricata* l. Int J Pharm Bio Sci 2013; 4(1): 389 – 396.
12. Kadir FA, Kassim NM, Abdulla MA and Yehye WA. PASS-predicted *Vitex negundo* activity: antioxidant and antiproliferative properties on human hepatoma cells-an in vitro study. BMC Complementary and Alternative Medicine 2013; 13: 343 1-13.
13. Kannikaparameswari N and Indhumathi T. Haematological and cytotoxic effect of the ethanolic extract of *Vitex negundo.* Int. J. LifeSc. Bt & Pharm. Res 2013; 2(1): 247-253.
14. Xin H, Kong Y, Wang Y, Zhou Y, Zhu Y, Li D and Tan W. Lignans extracted from *Vitex negundo* possess cytotoxic activity by G2/M phase cell cycle arrest and apoptosis induction. Phytomedicine 2013; 20(7): 640-7.
15. Fair JR and Humphrey JL, Liquid-Liquid Extraction Process, Fifth Industrial Energy Technology Conference, 1983; 2:846-856.
16. Bjergaard SP, Rasmussen KE and Halvorsen TG, Liquid-Liquid extraction procedures for sample enrichment in capillary zone electrophoresis. Journal of Chromatography, 2000; 9(02): 91-105.
17. Blainski A, Lopes GC, and De Mello JCP, Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium brasiliense* L. Molecules 2013; 18: 6852-6865.
18. Apak R, Gorinstein S, Bohm V, Schaich KM, Ozyurek M and Guclu K, Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure and Applied Chemistry 2013; 85(5): 957-998.
19. Zachariah ZM, Aleykutty N A, Jaykar B , Vishwanand V and Halima OA, Evaluation of antioxidant and total Flavonoid content of *Mirabilis Jalapa* Linn using *invitro* models, Int Res J Pharm 2012; 3(3):187-192.
20. Rajesh MP and Natvar JP, *In vitro* anti-oxidant activity of coumarin compounds by DPPH, Super oxide and Nitric oxide free radical scavenging methods. J Adv Pharm Ed Res 2011; 1: 52-68.
21. Patel PR and Patel N. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and Nitric oxide free radical scavenging methods, J Adv Pharm Eduand Res 2011; 1:52-68.
22. Selvakumaran M, Pisarcik DA, Bao R, Yeung AT and Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. Cancer Research 2003; 63: 1311-1316.
23. Raghavendra H. Lakshmanashetty, Vijayananda B. Nagaraj, Madhumathi G.H and Vadlapudi Kumar. *In* vitro Antioxidant Activity of *Vitex negundo* L. Leaf Extracts. Chiang Mai J. Sci. 2010; 37(3): 489-497.
24. Shah S, Dhanani T and Kumar S. Comparative evaluation of antioxidant potential of extracts of *Vitex negundo*, *Vitex trifolia*, *Terminalia bellerica*, *Terminalia chebula*, *Embelica officinalis* and *Asparagus racemosus*. Innovations in Pharmaceuticals and Pharmacotherapy 2013; 1(1): 44-53.
25. Huang HC, Chang TY, Chang LZ, Wang HF, Yih KH, Hsieh WY and Chang TM. Inhibition of melanogenesis versus antioxidant properties of essential oil extracted from leaves of *Vitex negundo* Linn and chemical composition analysis by GC-MS. Molecules 2012; 17(4):3902- 3916.
26. Murali krishna. T , Meena. G , kavya. T , Someshwar. C , Soumya. J, Aswaq ahmed, Vadluri R and Gajula RJ. *In vitro* determination of anti-oxidant and anti-bacterial activities of *Vitex negundo* linn. Int J Pharm Bio Sci 2013; 4(1): 121 – 127.
27. Zargar M, Azizah AH, Roheeyati AM, Fatimah AB, Jahanshiri F and Pak-Dek MS. Bioactive compounds and antioxidant activity of different extracts from *Vitex negundo* leaf. J Med Plants Res 2011; 5(12): 2525-2532.
28. Salleh RM, Dashti NG and Thong OM. Proximate Analysis and Anti-Proliferative Properties of *Vitex negundo* L. Sains Malaysiana 2014; 43(10): 1543–1547.

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