Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 389 - 396

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EVALUATION OF PHYTOCONSTITUENTS, ANTIBACTERIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF VITEX NEGUNDO L. AND TABERNAEMONTANA DIVARICATA L.

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ABSTRACT

The aim of present study was to evaluate antioxidant and antibacterial activity of *Vitex negundo* L.and *Tabernaemontana divaricata* L.Methanolic extract of *V. negundo* L. was found to contain142.96 ± 6.73 mg GAE/gm dry weight of phenolics and167.88 ± 4.80 mg QE /gm dry weightflavonoids. The leaf extracts of *V. negundo* L.demonstrated higher antioxidant activity as compared to *T. divaricata*L. Both the plant extracts showed broad spectrum antibacterial activity. The cytotoxic activity of theextracts of *V. negundo* L.and *T. divaricata* L. were assessed on THP-1 leukemia cell line using MTT assay and both the plants demonstrated cancer cell inhibitory activity.Both these shrubs have potential applications in pharmaceutical industry and medicine due to their phytoconstituents, antioxidants, antibacterial and anticancer activities.

KEYWORDS: ***Vitex negundo*L.,*Tabernaemontana divaricata* L., Antioxidant, antibacterial activity, Anticancer**

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive numbers of drugs have been isolated from natural sources[1]. Pharmaceutical importance of medicinal plants is due to specific constituents of secondary metabolites present in them[1].Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants and one of such resource is folk medicines[2]. World plant biodiversity is the largest source of herbal medicines and still about 60-80% world population rely on plant based medicines which are being used since the ages as traditional health care systems[3]. Though the recovery is slow, the therapeutic use of medicinal plants is becoming popular because they do not cause side effects and combat antibiotic resistant micro-organisms[4]. Antioxidants are agents which scavenge free radicals and prevent damage caused by reactive oxygen species (ROS)[5]. Several medicinal plants have been shown to exhibit potential antioxidant activity due to presence of various phytochemicals, e.g. flavonoids, phenolics, tannins, carotenoids [6]. Chemotherapy is one of the most common treatments for cancer which is caused by stress and carcinogens[7]. However, chemotherapeutic agents have many side effects. Herbal medicines have an important role in prevention and treatment of cancer with minimum side effects. *Vitex negundo* Linn. belongs to the family Verbenaceae is distributed in all over India[8]. It is an erect shrub, growing from 2-8 meter in height.The study of antibacterial activity of petroleum ether, chloroform, ethanol, methanol and aqueous extract of *V. negundo* L. *against* human pathogenic bacteria was done[9]. *In vivo* and *in vitro* anticancer activity study of ethanolic extract of *V.negundo* L. demonstrated the antitumor effect of ethanolic extract against Daltons Ascitic lymphoma (DAL) in swiss albino mice[10]. *Tabernaemontana divaricata* L. is a glabrous, evergreen dichotomously branched shrub belonging to the family Apocynaceae[11]. *T. divaricata* L. possess a wide range of therapeutic activities like alexipharmic, astringent, anticancer, hepatoprotective, digestive and antibacterial properties.[11] The ethyl acetate fraction of *T. divaricata* L. possesses potent antioxidant compounds[12]. Antibacterial activity of various solvent extracts of *T. divaricata* L. by disc diffusion method demonstrated that plant extracts were bacteriostatic at low concentration and bactericidal at higher concentration[13].

MATERIALS AND METHODS

1. Collection of plant material

Fresh leaves of *V.negundo* L. and *T. divaricata* L. were collected from and around Modern College, Pune-05 campus and authenticated by Dr. Neeta Patil, Botany, Modern College, Pune. The leaves were washed twice with distilled water, dried and powdered.

2. Preparation of extract

Methanol and acetone extract was prepared according to method of Vaghasiya *et al*[14].10 gm dried powder of each plant material was extracted first with petroleum ether and then with respective solvents including methanol and acetone. For ethanol extract, 10 gm air dried powder of plant material was extracted in 200 ml absolute ethanol by soxhlet extraction. Aqueous extract was prepared by adding 10 gm of plant powder to 100 ml distilled water and by incubating the flask in water bath at 55°C.The solvent was evaporated under vaccum and dry extract of each plant material was dissolved in appropriate amount of respective solvent.

3. Qualitative phytochemical analysis of V.negundo L. and T. divaricata L.

Preliminary phytochemical analysis of various extracts of *V.negundo* L. and *T. divaricata* L.was done qualitatively to detect the presence of various phytoconstituents. The tests were performed to detect phytochemicals viz. Tannins

($FeCl_3$ test), Alkaloid (Dragendorff's test), Reducing sugars (Fehling's test), Protein (ninhydrin test), Steroids and triterpenoids (Salkowski test) and Saponin (froth test) [3].

4. Estimation of Total Phenolic Content

The total phenolic content in various extracts of *V.negundo* L. and *T. divaricata* L. was determined by method of Raghavendra[15] and Slinkard[16], with some modifications. 10 µl of plant extract was added to 490 µl distilled water to which 2.5 ml Folin- Ciocalteau reagent (SRL) and 2 ml of 7.5% Na_2CO_3 was added. The reaction mixture was incubated for 90 min at room temperature and absorbance was recorded at 750 nm. Gallic acid was used as standard.

5. Determination of total flavonoids

Total Flavonoid content in various extracts was determined by the methodof Raghavendra[15]and Zhishen[17]with modifications. 50 µl of plant extract was added to 4950 µl Distilled water and was mixed with 0.3 ml of 5 % $NaNO_2$. This was incubated for 5 min at room temperature and 0.3 ml of 10 % $AlCl_3$ was added to the mixture. After 6 min of incubation 2 ml of 1M NaOH was added to the mixture followed by addition of 2.4 ml distilled water. Absorbance was recorded at 510 nm. Querscetin was used as standard.

6. Determination of total antioxidant capacity by phosphomolybdenum method

The antioxidant activity of various plant extracts was determined by method of Raghavendra[15] and Prieto[18], with some modifications.10 µl plant extract was added to 290 µl distilled water was mixed with 3 ml of reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The reaction mixture was incubated at 95° C for 90 min and after cooling, absorbance was recorded at 695 nm. L-Ascorbic acid was used as standard.

7. Evaluation of antibacterial activity

The antibacterial activity of *V.negundo* L. and *T. divaricata* L. was tested against *Bacillus cereus, Escherichia coli, Staphylococcus aureus,* and *Klebsiella sp*. The antibacterial activity was assessed by seeding 0.1 ml of test bacterial culture (Optical density at 600 nm = 0.5) on MullerHinton agar plates (Hi Media). 5 mm wells were made on agar surface with 5mm cork borer to which 20 µl of each plant extract was added. Plates were incubated at 37°C for 24 hrs. and zone of inhibition was measured.

8. Cytotoxic activity of plant extracts

The cytotoxic activity of extracts of *V .negundo* L. and *T. divaricata* L. on THP-1 leukemia cell line was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazoliumbromide) assay[19]. The THP-1 cell culture was purchased from National Centre for Cell Sciences, Pune. They were cultured in Roswell Park Memorial Institute 1640 medium (RPMI) (Himedia®) with 10% Fetal Calf Serum (FCS). THP-1 (Acute Myeloid Leukemia cell line) cells were seeded in 96-well plates at a density of 1.0×10^4cells/well. It was then incubated overnight at 37°C in a 5% CO_2 humidified environment. 20 µl of different solvent extracts of *V.negundo* L. and *T. divaricata* L. were added to wells. After incubation for desired period of time at 37°C in humidified incubator, cell viability was assessed by MTT assay. The cells were incubated with MTT for 4 hrs, they were centrifuged, MTT was removed and 100 µl DMSO containing 25 µl Glycine buffer was added to each well. Absorbance was measured at 595 nm on micro plate reader (i mark™). The experiment was carried out in triplicates and percentage of growth inhibition was calculated as follows: Growth Inhibition rate (% = [Absorbance of control – Absorbance of test/ Absorbance test] x 100.[19] The above formula gives % viability using which % inhibition was calculated.

RESULTS AND DISCUSSION

1. Qualitative phytochemical analysis of V.negundo L. and T. divaricata L.

Qualitative tests of plant extracts of *V.negundo* L. and *T. divaricata* L. were performed to detect the presence of various phytochemicals including Alkaloids, Tannins, Reducing sugars,

Saponin, Steroids and triterpenoids and Proteins. Bioactive compounds like Steroids, Alkaloids, Tannins, Reducing sugars, proteins were present in the solvent extracts. Alkaloids and tannins were present abundantly in almost all the extracts. Ethanol, methanol and acetone extracts were found to contain various phytochemicals as compared to aqueous extract of plants.

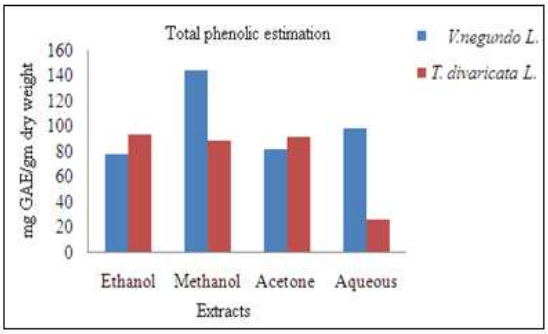
Table 1
Preliminary phytochemical screening

	Vitex negundo L.				*Tabernaemontana divaricata* L.			
	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol	Acetone	Aqueous
Alkaloids	+	+	+	-	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Steroid	-	-	-		+	+	-	-
Tri-terpenoid	+	+	+	+	-	-	+	+
Saponin	+	+	-	+	+	+	+	+
Protein	-	+	+	+	+	+	+	+
Reducing sugars	+	+	-	+	+	+	+	+

2. Total phenolic estimation

Phenolic compounds are present in both edible and non-edible plants and their parts and have multiple biological effects. The antioxidant property of phenolics compound is mainly because of their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.Total phenolic content was assayed by Folin-Ciocalteau method. The content of phenolics compounds was determined from regression equation of calibration curve ($y=0.022x–0.043$, $R^2=0.993$) and expressed in Gallic acid equivalents (GAE). Among the 2 plants evaluated for total phenolics content, the methanolic extract of *Vitex negundo* L. showed highest amount of phenolicsi.e.142.96 ± 6.73 mg GAE/ gm dry weight of plant material.

Graph 1
Total phenolics content

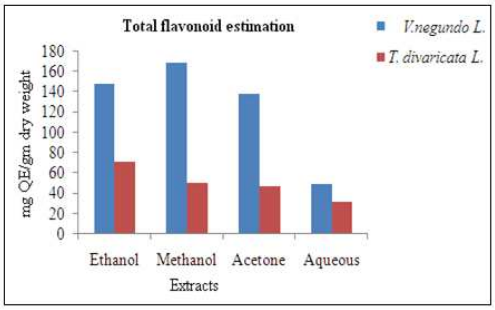


3. Total flavonoid estimation

Flavonoids are potent antioxidants therefore are of main interest as they are involved in protection against cardiovascular diseases.[15]The content of flavonoids was determined from regression equation ($y=0.003x+0.009$, $R^2=0.995$ and $y=0.002x+0.051$, $R^2=0.993$) of calibration curve and expressed in Querscetin equivalents (QE). Among both the different plants tested for total

flavonoids content, ethanolic, methanolic and acetone extract of *Vitex negundo* L. showed highest amount of flavonoids which was 147.25 ± 7.65 mg QE/gm DW, 167.88±4.80 mg QE/gm DW, 137.19±1.98mg QE/gm DW respectively. Extracts of *T. divaricata* L. was found to possess considerable amount of flavonoids. As compared to other solvent extracts, aqueous extract showed lesser amounts of flavonoids.

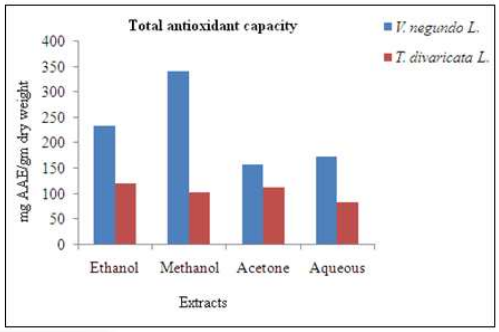
Graph 2
Total flavonoid content



4. Estimation of total antioxidant activity

The phosphosmolybdenum method used for total antioxidant activity is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximum absorption at 695 nm. [15] The antioxidant activity was determined by the regression equation of calibration curve (y = 0.002x+0.023, R^2=0.981) and expressed as ascorbic acid equivalents (AAE). Among both the plants, the methanolic extract of *V.negundo* L. showed the highest antioxidant activity i.e. 341.66±13.60 mg AAE/gm dry weight of plant material followed by the ethanolic extract of the same plant i.e. 233.33±.2.95 mg AAE/gm DW. The extracts of T. *divaricata* L. were found to possess considerable antioxidant capacity. The antioxidant activity of plant extracts may be due to their phenolics and flavonoid contents. It is known that the ethyl acetate fraction of *T. divaricata* L. possesses potent antioxidant compounds[12].

Graph 3
Total antioxidant capacity

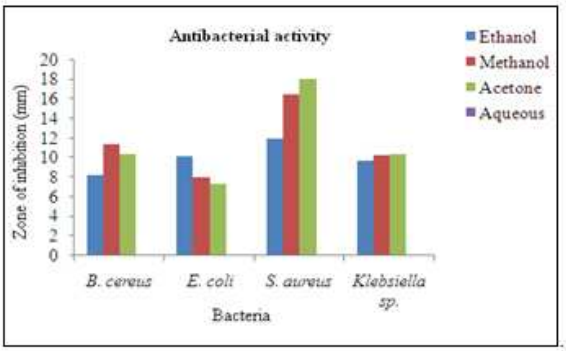


5. Evaluation of antibacterial activity

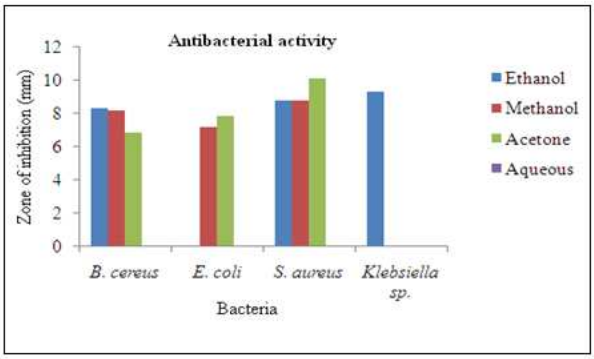
Different solvent extracts of *Vitex negundo* L. and *Tabernaemontana divaricata* L. were tested against Gram positive and Gram negative bacteria including *Bacillus cereus, Escherichia coli*, *Staphylococcus aureus*, and

Klebsiella sp. The antimicrobial activity was determined agar well diffusion method and the zone of inhibition was recorded. Ethanolic, methanolic and acetone extracts of *V. negundo* L. showed effective antibacterial activity against all the bacteria with highest activity against *S. aureus. T. divaricata* L. was also found to be effective against almost all the bacteria. Aqueous extract of both plants showed no antibacterial activity. Panda et al, have also reported that all the extracts of *V. negundo* L show complete inhibition of *Escherichia coli* and ethanol and methanol extracts of leaves were found to be most effective inhibiting agent against both Gram positive and Gram negative bacteria.[9]

Graph 4
Evaluation of antibacterial activity of V. negundo L.



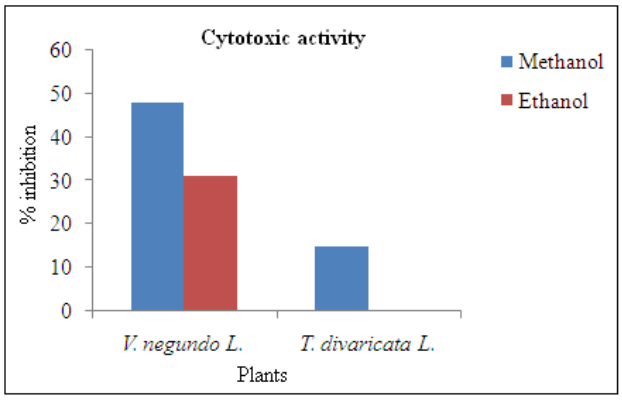
Graph 5
Evaluation of antibacterial activity of T. divaricata L.



6. Cytotoxic activity of plant extracts

The evaluation of cytotoxic activity is based on quantification of purple colored formazan, which is formed by the reduction of MTT [3-(4,5-dimethylthiazole-2-yl))-2,5-diphenyl tetrazolium bromide]. The reduction of MTT is proportional to the number of active mitochondria in the living cells[19]. The cytotoxic activity of *Vitex negundo* L. and *T. divaricata* L.was assessed against THP-1 leukemia cell line. The methanolic extract of *Vitex negundo* L. showed maximum growth inhibition rate of 48.14±0.92 % against THP-1 cell lines, followed by ethanolic extract of *Vitex negundo* L. (31.13±2.64%). Ethanolic and methanolic extract of *T. divaricata* L showed less activity.

Graph 5
Cytotoxic effect of plant extracts against THP – 1 cancerous cell line



Vitex negundo L. and *T. divaricata* L have been assessed for their antimicrobial, antioxidant and anti cancer activities. Both the shrubs demonstrate potential for application in pharmaceutical industry due to their photochemical properties.

CONCLUSION

The data obtained from the present study suggest the presence of various phytochemicals in the different solvent extracts of *Vitex negundo* L. and *T. divaricata* L. Total phenolic content was higher in *Vitex negundo* L. methanolic extract and that was lower in *Tabernaemontana divaricata* L. aqueous extract. Flavonoids content was also found to be higher in methanolic extract of *Vitex negundo*L.This extract contains an effective antioxidant fraction that has been confirmed by phosphosmolybdenum method i.e. total antioxidant capacity. Antimicrobial activity of plant extract has been shown that *Vitex negundo* L. and *T. divaricata* L. has a broad spectrum of activity which can be used as leads in developing the novel therapeutic bioactive agents. *In vitro* growth inhibitory property of these plants supports to exert their anticancer effects *in vivo*. Thus it can be concluded that both the shrubs *Vitex negundo* L. and *T. divaricata* L. have immense potential for applications in pharmaceutical industry and medicine due to their phytoconstituents, antioxidants, antibacterial and anticancer activities.

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