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Study on in vitro antioxidant activity, antibacterial and anticancer activities of Vitexnegundo-Nochi

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ABSTRACT
Phytochemicalsareecologicallyderivedplantsecondarymetabolitesthatplantsmaketoprotectthemselvesagainstenvironmentalstressandpathogenicmicrobialinvasion.Thesephytochemicalshavebeenshowntohavebothpositiveandpharmacologicaleffectsinthetreatmentofhumanillnesses.Itiswellknownthattheactiveprinciplesfoundinmedicinalplantsworktogethertoalleviatetheprimaryandsecondarycomplicationsofavarietyofdiseases.ForthetreatmentofCovid-19,VitexnegundoL.(Verbenaceae)isfrequentlyutilizedasamedicinalagentaswellasadietarysupplement.In thisstudy,theabilityoftheethyl acetateextractVNtoscavengefreeradicalswasassessedusingDPPHassays,andVNEAwasinvestigatedforin vitroanticanceractivityagainstbreastcancercellsforanticanceractivityassessment.Theantibacterialactivityintheevaluationofplantextractsperformedagainst S.aureus,E.coli,K.pneumoniaeandP.aureginosabydiscdiffusionmethod.Theresultsobtainedevidencedthattheethyl acetateextract(VNEA)possessessignificantantioxidant,antibacterialandanti-canceractivitythedatapresentedprovidescientificevidencefortheantioxidantandanticancertherapeuticefficacyofthemedicinalplant,whichinturnmaybeduetothepresenceofbiologicallyactivemoleculespresentintheherbal.

Keywords: Vitexnegundo,invitroantioxidantassays, anti-bacterial

I. INTRODUCTION

Plants used in traditional medicine represent a priceless tank of new bioactive molecules. Vitexnegundo L. is one of the important plant from traditional system of medicine found all over the world [1]. Vitexnegundo L is large and erect aromatic shrubs grow to height 3–6 m or slender tree with quadrangular branchlets distributed throughout India. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 5–11 cm long, hairy beneath and pointed at both ends [2]. The bluish purple flowers are numerous. The fruit is succulent, black when ripe, rounded and about 4 mm in diameter [3].

The various chemical constituents present in leaves of VitexnegundoLinn leaves are Friedelin, Vitamin-C, Carotene,casticin, artemetin, terpinen-4-ol, α-terpineol, sabenine, globulol, spathulenol, β- farnesene, farnesol, bis (1,1dimethyl) methylphenol, α-pinene, β-pinene, linalool, terpinyl acetate, caryophyllene epoxide, caryophyllenol, vitexicarpin, viridiflorol, 4,4′′- dimethoxy-trans-stilbene, 5,6,7,8,3′4′5- heptamethoxy, 5-hydroxy-6,7,8,3′4′pentamethoxy(5-Odesmethylnobiletin),5-hydroxy-6,7,8,3′,4′,5-hexamethoxy(gardeninA), 5-hydroxy-6,7,8,4′tetramethoxy (gardeninB), 5-hydroxy-7,3′,4′,5′-tetramethoxyflavone (corymbosin), terpinen-4-ol, αcopaene, β-caryophyllene, β-elemene, camphene, α-thujene, α-pinene, sebinene, linalool, stearic acid and behenic acid, α-elemene, δ- elemene, β-elemene [4-7]. The seeds of Vitexnegundo Linn have chemical constituents such as n-Tritriacontane, n-hentriacontanol, nhentricontane, n-pentatricontane, n-nonacosane, β-sitosterol, phydroxybenzoic acid and 5-oxyisophthalic acid, 3, 4-dihydroxybenzoic acid, artemetin, 3β-acetoxyolean-12-en-27-oic acid, 5β-hydro-8,11,13-abietatrien6α-ol, 2α,3α-dihydroxyoleana-5,12-dien-28-oicacid, 2β,3α-diacetoxyoleana-5,12-dien-28-oicacid and 2α,3βdiacetoxy-18-hydroxyoleana-5 [8-10]. The various chemical constituents present in the stem and bark are 3,6,7,3′,4′-Pentamethoxy-5-Oglucopyranosylrhamnoside, vitexincafeate, 4′-O-methyl myricetin- 3-O-[4′-O-β-D-galactosyl]-β-D-galactopyranoside, βamyrin, epifriedelinol and oleanolic acid, Hepta methyl-phenyl-cyclotetra siloxane, Cycloheptasiloxane,tetradecamethyl Nona methyl, phenyl-cyclopenta siloxane, Cyclooctasiloxane,hexadeca methyl, Borazine, 2,4,6-tripheny-l1, 3, 5-tryophl, Nonamethyl, phenyl-cyclopenta siloxane. Vitexoside, agnuside, R-dalbergiphenol, negundin A, negundin B, 6-hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaledehyde, vitrofolal E, (+)-lyoniresinol, (+)-

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lyoniresinol-3α-O-βd-glucoside, (+)-(−)-pinoresinol, and (+)-diasyringaresinol [11-14].

Inthispresentstudy,theabilityoftheethyl acetateextract(VN EA)scavengingfreeradicalswasassessedbyusingDPPHassays,invitroanticancer activity against breast cancer cells.Theantibacterialactivityfortheevaluationofplantextracts Performedagainst S. aureus, E. coli, K. pneumoniaeand P. aureginosabydiscdiffusionmethodstoexploretherapeuticpotential.

TAXONOMICAL CLASSIFICATION

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyte
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Verbenaceae
Genus	Vitex
Species	Negundo

Figure1: PhotographshowingVitexnegundoL.

II. MATERIAL & METHODS

Collectionofthesample

Forthisstudy,VitexnegundowasgatheredfromAravindhherballaboratoryinRajapalayam,Tamilnadu(Figure1).

Methodofpreparationofsample

10gVitexnegundoand100mlethyl acetateisheatedseparatelyfor5hoursinawaterbathwitharefluxcondenser,thencooledandfiltered.Ethyl acetateextractwas obtainedbyvacuumevaporationofthefiltrate(VN-EA).

Pharmacologicalevaluation

Invitroantioxidantactivity

Vitexnegundoethyl acetateextract[VN EA]investigatedforinvitroantioxidantactivitybyDPPHethyl acetateextract[15].

DeterminationofDPPHradicalscavengingactivity

Brand-Williamsetal.(1995)[16]usedDPPH(1,1-Diphenyl-2,Picryl-Hydrazyl)freeradicalstoassessantioxidantactivityinthesampleVNforfreeradicalscavengingactivity. Inthemicrotitreplate,100µlofVNEA extractwasused.100µlof0.1percentethyl acetateDPPHwasappliedtothesamplesandincubatedinthedarkfor30minutes.Thesampleswerenextexaminedfordiscoloration;strongandweakpositivesweredefi

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nedaspurpletoyellowandpalepink,respectively.With Standardascorbicacidasareference,readtheplateat490nmonanElisaplatereader.Allofthetestsweredoneintriplicate,andmeanvalueswerecalculated. Radicalscavengingactivitywascalculatedbythebelowmentionedequation:
DPPHradicalscavengingactivity(%)=[(Absorbanceofcontrol-Absorbanceoftestsample)/(Absorbanceofcontrol)]x 100.

Invitroanticanceractivity

Theanticanceractivityof(VN-EA)extractwastestedusingtheMTTassayagainstbreastcancercell line(MCF-7cells) [17].Forthepredictionsofanticanceractivityandcytotoxicitypotentialofthepreparedextracts,parameterssuchasinhibitoryconcentration(IC50-concentrationrequiredtoinhibitthegrowthof50% cancercells)andcytotoxicity(CC50-concentrationrequiredtoinhibitthegrowthof50% normalcells)weremeasured.Table2showtheanticanceractivityandcytotoxicitydata.

Antibacterialactivity

Thediscdiffusionmethodwasusedtotesttheantibacterialactivityofplantextractsagainst*S. aureus, E. coli, K. pneumoniae*and *P. aureginosa*.Thebacterialcultureswereusedtomakethe microorganism'sinoculum.Incleansterilizedpetridishes,15mlofnutrientagar(HiMedia)mediumwasput.Allowtimeforittocoolandharden.Around100µlofbacterialstrainbrothwaspipetteoutandevenlydispersedoverthemediumwithaspreadingroduntilitdried.Asterilecorkborerwasusedtodrill6mmdiameterwells.AlloftheextractsweredissolvedinDMSOataconcentrationof1mg/ml.Thewellswerefilledwith100µlofVNextractssolutions.Thepetriplateswereincubatedfor24hoursat37°C.Apositivecontrolofstreptomycin(1mg/ml)wasused,whileanegativecontrolofDMSOwasused.Thediametersofthezoneofinhibitions(ZI)weremeasuredtodetermineantibacterialactivity,andallmeasurementsweredoneintriplicate [18].

III. RESULTS & DISCUSSION

TheantioxidantactivityofVitexnegundoextract(VN-EA)wasexaminedinvitrousingtheDPPHtechniquewithascorbicacidasthestandard.Whencomparedtoascorbicacidunderidenticalconditions.VN-EAextractdemonstratedsubstantialanti-oxidantactivity(Table.1).Ethyl acetateextractofVN-EAwasexaminedforanticanceractivityinvitroagainst breast cancercells.VN-EAexhibitedanti-canceractivity(IC_{50}45.63µg/ml)againstbreastcancer cells (Table 2).

Table 1: In vitro antioxidant activity of VN EA by DPPH assay

S. No	OD (nm)	COD	SOD	Percentage of Inhibitions (mg/ml)	Average (mg/ml)
VN - EA					
1	520	0.99	0.08	91	71
2	520	0.99	0.09	90	
3	520	1.0	0.23	67	
4	520	0.99	0.27	72	
5	520	0.99	0.23	66	
6	520	0.99	0.61	38	
Ascorbic acid					
7	520	0.73	0.04	94.5	93.5
8	520	0.73	0.06	91.7	
9	520	0.73	0.04	94.5	

Table2: EffectsofcompoundagainstMCF-7CelllinebyMTT assay

S. No.	Sample	Concentration (µg/ml)	OD	% inhibition	IC_{50}
1	Control		1.538		
2	Std. 5 FU	10	0.731	52.47	37.56
		40	0.673	56.24	
		100	0.633	58.84	

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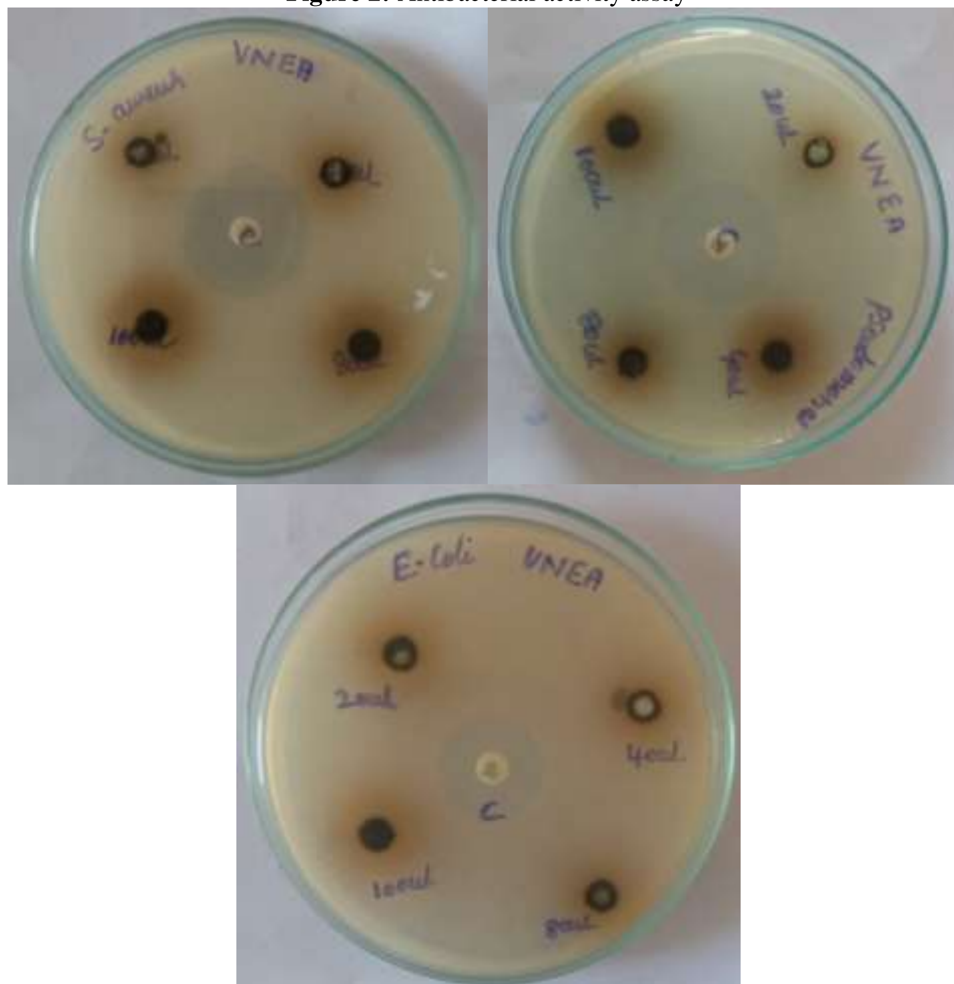
2	VN-EA	10	0.401	73.92	45.63
		40	0.378	75.42	
		100	0.329	78.60	

IC_{50} – 50% inhibitory concentration

Table 3: In vitro antibacterial activity by disc diffusion method

S. No.	Organism	Zone of Inhibition mm	
		VN EA	Streptomycin (Std.)
1	S. aureus	12mm	26 mm
2	E. coli	10 mm	19 mm
3	K. pneumoniae	….	….
4	P. aureginosa	12 mm	25 mm

Figure 2: Antibacterial activity assay



Inthisstudy,ethyl acetate(VN-EA)extract(VN-EA)testedforinvitroantioxidantactivity andanticanceractivity.Evaluationofinvitroantioxidantpropertiesstudiedby DPPHassayofthegivensamples wasassayedusingascorbicacidasthestandardVNEA extractshowngoodactivity.(Table1).VN EA exhibited significant anticancer activity against breast cancer cells (MCF-7) (IC_{50} 45.63µg/ml) when compared to the standard 5-Fluro Uracil (IC_{50} 37.56µg/ml).

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VNEAhadsignificantantibacterialactivityagainst S. aureus and P. aureginosa(Table3andFigure2).

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