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Uvphotoreactor techniques and Arima Modeling in the comparison of antioxidant potential of Medicinal Plants

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Abstract
In this research work, phytochemical analysis of Curcumin ,Vetiveriazizanioides and Piper nigrum were carriedout by using water and acetone as solvents by greener Sonication method.Ultrasound sonication is one among thebest extraction methods in which frequency range of 20 kHz, electrical sound energy transformed into physicalvibration mode agitated the solid plants at 70^0 c and disrupts the cellular chemical compounds into solution withoutdamagingtheoriginalnature.AqueousextractsofVetiveriazizanioidesshowedthepositiveresultsforCarbohydrates, alkaloids, flavonoids, Coumarin, Quinone and terpenoids. But Polyphenol was not found in aqueousextract ofVetiveriazizanioides. Bothaqueous andacetone extracts ofPiper nigrumshowedthe positive resultsforFlavonoids,Coumarin,QuinoneCarbohydrates,alkaloids,terpenoidsandPolyphenolcompoundsTheconte ntoftotal phenoliccompounds wasdeterminedspectrometricallyand antioxidantpropertyhas beencalculatedbyusing ascorbic acid as a reference standard. Copper II neocuproine was prepared and used to measure the anti oxidant potential of phytocompounds. Along with [Cu Ncu]$^{2+}$ all the extracts were taken in UV photo reactor flask and analyzed for every 5 minutes interval. Absorption peaks were noted and Antioxidant property of these extracts were identified inthe increasing order of the λ_{max}plants as aqueous extracts of Vetiveriazizanioides, aqueous extracts of Pipernigrum acetone extract of Vetiveriazizanioides acetone extracts of Piper nigrum the same extracts as above forcurcumin were also analyzed. Curcumin showed better result when compared to all other extracts. To develop thesesignificant sources of natural antioxidants, further extraction with different organic solvents and characterization ofthe phenolic compositionisneeded.

Keywords: *Copper II neocuproine, Phytochemical analysis, antioxidant potential, Curcumin ,Vetiveriazizanioides and Piper nigrum*

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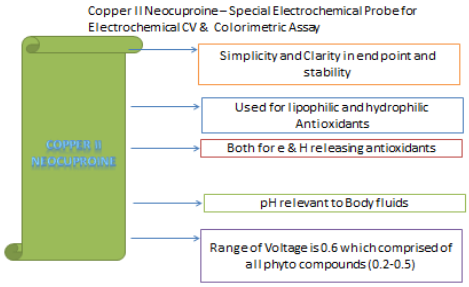
I. Introduction

Antioxidants are chemical compounds that inhibit oxidation, a chemical reaction that can produce free radicals andchain reactions that may damage the cells of organisms. Primary antioxidants, acted as free radical terminators(scavengers); secondary antioxidants, which are important preventive antioxidants that function by retarding chaininitiation. Natural plants possessed large number of phyto compounds which were acting as anti-oxidants in ourbody.*Curcumin*,*Vetiveriazizanioides*andPipernigrumshowedbetteranti-oxidantbehaviourinliteraturesurvey.Preparationofwaterandacetoneextractsof*Vetiveriazizanioides*and*Pipernigrum*Carbohydrates,alkaloids,flavonoids,Coumarin,Quinoneandterpenoids.InthisworkPhytochemicalanalysisofCurcumin,*Vetiveriazizanioides*and*Pipernigrum*wereunderwentusingUltrasoundsonicationmethodgreenerforextraction.Theirantioxidantpotentialswerefoundandarranged asa new orderforbetterunderstanding.
Copper II neocuproine complex has the following importance and used to measure Antioxidant potential of



Phytocompounds.

COLLECTIONOFPLANTS

The whole plants were washed under running tap water, shade dried at room temperature, and powdered.Thepowderedplantsample(50g/250ml)wasextractedsuccessivelywithpetroleumether,acetone,chloroform, ethanol and water usingSonicator and Soxhlet apparatus at 55-850°C for 8-10 hrs to extract the polar andnon-polar compounds [4]. For each solvent extraction, the powdered pack material was air dried and thenused.

PREPARATIONOFPLANTEXXTRACT

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolitespresentinpetroleumether,acetone,chloroform, ethanol andaqueousextract of wholetest plants.

GREENERULTRASONICATIONTECHNIQUE

Ultrasound sonication is one among the best methods in which frequency range of 20 KHz electricalsoundenergytransformedintophysicalvibrationmodeandactedonthesurfaceofthechemicalcompounds.Itagitatedthesolidplantsatroomtemperatureanddisruptedthecellularchemicalcompounds into solution without damaging the original nature. Chemical reagents for phytochemicalanalysiswerereactedwiththechemicalcomponentspresentintheplantextractsandgavethecharacteristic coloured solutions. Various oxidation and reduction reactions were carried out with thefunctionalgroups possessed in phytochemicalcomponents.

PHYTOCHEMICALSCREENINGTEST

Testforterpenoids:

An amount of 5ml of flower extracts was taken in a test tube, and then poured 10 ml of methanol in it,shaken well and filtered .Then 2 ml of chloroform were mixed in extract of TP and 3 ml of sulphuric acidwere added in selected sample extract. Formation of reddish brown colour indicated the presence ofterpenoidin the selected extracts.

TestforFlavonoids:

1. Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993;Harbrone, 1973).5 ml ofdilute ammonia solutionwere addedto a portionoftheaqueousfiltrateofeachplantextractfollowedbyadditionofconcentratedH_2SO_4.A yellow colouration observed ineachextractindicatedthe presence of flavonoids.

2. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration wasobservedindicating the presenceofflavonoids.

3. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over asteam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of diluteammoniasolution.Ayellowcolourationwasobservedindicatingapositivetest forflavonoids.

Testforalkaloids

An amount of 3 ml of the selected flower extracts were added in each test tube and 3 ml of hexane weremixed

in it, shaken well and filtered. Then took 5 ml of 2% HCl and poured in a test tube having themixture of plant extract and hexane. Heated the test tube having the mixture, filtered it and poured a fewdropsofpicric acid in a mixture.Formation ofyellow colourprecipitate indicates thepresenceofalkaloids

Molischtestforcarbohydrates
3 ml of each extract of the two samples was treated with alpha-naphthol and 3 drops of con.H 2 SO .Theformationof violetcolouration confirmedthepresenceof carbohydrate.
Keller-killanitestforCardiacGlycosides:
3 drops of each extract was treated with 1.5ml of glacial acetic acid and 1drop of 5% ferric chloride anddaddedconcentrated H 2SO4along the sides of the test tube. The blue coloured solution suggested thepresenceof cardiacglycosides.
XanthoproteictestforProtein&Aminoacids:
Few dropsofconcentratednitricacidwereaddedtothisextract,yellow colourappearedinthesolution.Itreflectedthepresence of protein &aminoacid.
FerricchloridetestforPhenoliccompounds:
The extract was treated with few drops of 5% ferric chloride. The colour changed from dark green tobluishblackwhichrecommendedthe presence ofphenolic compounds.
AceticanhydridetestPhytosterols:
2mLofaceticanhydrideand2mLofconcentratedsulphuricacidwastreatedwiththeextract,Thevioletcolouredsolution changed into green whichshowedthe presence ofphytosterols.
ConcentratedHCl acidtestforQuinones
Theplantextractwastreatedwithconcentrated HCl.Thedarkgreencolouredsolutionturnedintolightgreencoloured solution, this desiredthe presence of quinones.
Effervescencestest ForCarboxylicacid
Anamountof1mLofplantextractwasaddedwithsodiumbicarbonate.Theappearanceofeffervescenceconfirmedthepr esence of carboxylic acid.
SodiumhydroxidetestForCoumarins
Theextractwastreatedwith10%NaOHandchloroform.Appearanceofayellowcoloursolutionindicatedthe presence ofcoumarins.
Detection of PhytosterolsLibermannBurchardtest
Theextractwastreatedwith2mLaceticanhydridefollowedbyconcentratedH_2SO_4,anarrayofcolourchange wasobservedinthe solution.Itconfirmedthe presenceofphytosterol.
DetectionofCholesterol
The extractwastreatedwith2mLaceticanhydride and 2mLofchloroformfollowedbyconcentratedH_2SO_4 , Rose – redcolourwas observedinthesolution. Itconfirmedthe presenceofphytosterols.

The phyto chemical components were analyzed and the results were reported in the following table.1 .Inthis extract, important medicinal components such as cardiac glycosides, Alkaloid, quinone, coumarinswereidentified.

QUANTITATIVETESTS
Quantitative determination of the chemical constituencyPreparationoffatfreesample:2gofthesampleweredefattedwith100mlofdiethyletherusingasoxhletappar atusfor 2h.

Estimationofflavonoids
1. Totalflavonoidcontentsweremeasuredwiththe aluminum chloride colorimetric assay (Kumar etal.,2008)Aqueousandethanolicextractsthathasbeenadjustedtocomeunderthelinearityrangei.e.(400µg/ml)orAliqu ots ofextractsolutions weretaken andmade upthevolume 3mlwithmethanolanddifferentdilutionofstandard solutionofQuercetin (10-100µg/ml) were added to10ml volumetricflask.Totheabovemixture,0.3mlof5% $NaNO_2$wasadded. After 5minutes,0.3mlof 10% $AlCl_3$ was added. After 6 min, 2ml of 1 M NaOH was added and the totalvolume wasmade upto 10mlwith distillwater. Then the solution was mixed well and the absorbance was measuredagainsta freshly preparedreagent blank at520 nm. Total flavonoid content of theextracts wasexpressedaspercentageofQuercetin equivalentper100 g dry weightofsample.

2. FlavonoiddeterminebythemethodofBohmand Kocipai-Abyazan(1994).10goftheplantsamplewas extracted repeatedly with 100 ml of 80% aqueous methanol atroomtemperature.Thewholesolutionwasfiltered through whatman filter paper No 42 (125 mm). The filtrate waslatertransferredintoacrucibleandevaporatedinto drynessoverawaterbathandweighedtoaconstant weight. Determinationof totalphenolsbyspectrophotometric

method:Thefatfree samplewasboiledwith50ml ofether forthe extractionof thephenolic component for15 min. 5ml of theextract was pipetted into a50 mlflask, then 10 ml of distilled water was added. 2 ml of ammoniumhydroxide solutionand 5ml ofconcentrated amylalcohol were also added. The sampleswere made uptomarkandleft toreactfor30minforcolourdevelopment. Thiswasmeasuredat505nm.

DeterminationofAlkaloid
Alkaloiddetermineby themethodof Harborne (1973). 5 g of the sample was weighed into a 250 mlbeaker and 200ml of 10% acetic acidin ethanolwas addedandcovered and allowed to stand for 4 h.This was filtered andthe extract was concentrated on a water bath to one-quarter of the original volume.Concentrated ammonium hydroxide was addeddrop wise tothe extractuntil the precipitation wascomplete.The whole solution was allowed tosettleandtheprecipitatedwascollectedandwashedwithdilute ammonium hydroxide and then filtered. Theresidueisthealkaloid, which was dried andweighed.

DeterminationofRiboflavin
RiboflavinwasdeterminedasperthemethodgivenbyOkwu(2004).5gmsoftheindividualplant sample was extracted with 100 ml of 50% ethanol solutionand shaken for 1 hr. This was filteredintoa 100ml flask;10mlofthe extractwaspipetted into50mlvolumetricflask. 10 ml of 5% potassium permanganate and10 ml of 30%H2O2 wereadded andallowedto standover ahot water bath for about 30 min. 2 ml of 40% Sodium sulphate was added. This was made up to 50mlandthe absorbance wasmeasured at510 nmin aspectrophotometer.

TannindeterminationbyVan-Burden andRobinson (1981)method:
500 mg of the sample was weighed into a 50 mlplasticbottle.50mlofdistilledwaterwasaddedandshaken for1 hin amechanical shaker.This wasfilteredinto a 50ml volumetric flask andmadeupto themark.Then 5 mlofthe filtered waspipetted outintoa testtubeand mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferro cyanide. The absorbancewasmeasuredat120 nmwithin10min.DeterminationofSaponin:
The methodusedwasthatofObadoniand Ochuko (2001). The samples were ground and 20 g ofeach were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples wereheatedoverahotwaterbathfor 4 hwithcontinuousstirring at about55^{0}C.
The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. Thecombinedextractswere reducedto 40mloverwaterbath atabout90 ^{0}C. Theconcentrate was transferred into a 250 ml separatory funnel and20mlofdiethyletherwasaddedandshaken vigorously.Theaqueouslayerwasrecoveredwhilethe etherlayerwasdiscarded.Thepurificationprocess was repeated. 60 ml of n-butanol was added. The combined n-butanol extractswerewashedtwicewith10mlof5% aqueoussodiumchloride.The remaining solution was heatedina water bath.After evaporationthesampleswere dried intheovento aconstant.

DeterminationofAscorbicacid:
Ascorbic acid was determined as per the methodgiven byBarkat etal., (1973). 5gmof samplewastaken into100mlEDTA/TCA(2:1)andmixedwell.This mixturewascentrifugeat3000rpmfor20min.Itwas transferredto100mlvolumetricflaskandvolumewas made up. 20ml of thismixturewith1%starchsolutionwastitratedwith20%$CuSO_4$tilltheappearanceofdarkendpoint.
DeterminationofVitaminA
DeterminationofvitaminAbythemethodof (Bayfield andCole, 1980).Grind 1to5gm ofthesample materialto afinepasteandadd 1.0mlofsaponification mixture. Reflex the tubes gently for20minutes at 600C and cool the tubes at room temperature added 20mlwater and mix well. Extractvitamin with 10ml of petroleum ether in a separating funnel twice. Pool the extract and added sodiumsulphatetoremovethemoisturefor30-60minutes evaporate5mlaliquotoftheetherextracttodrynessat 60^{0}Cdissolve thedriedresiduein 1.0ml ofchloroform. Makeupthevolumeineachtesttubeto1.0mlwith chloroform.Added2.0mlofTCAsolutionfromafast delivery pipette,rapidlymixing the contents ofthetube.
Readat620nmimmediatelyinaspectrophotometer.

UVPHOTREACTORANALYSIS
In UV photo reactor, only the molecules possessed in high conjugation and free movement of electronslike Alkaloid, TerpinoidQuinone , flavonoid, Tannin, Ascorbic acid and Vitamin Ain Phyto chemicalsunderwent absorption category. Every 5 mits , the sample was taken from the reactor and λmaxwerecalculated.Alltheanalysedsamplesshowedabsorptionvaluesat different concentrationlevel.

Figure.1– UVphotoreactor

UVSPECTROPHOTOMETERCALCULATIONFORλ_{max}

PreparationofstandardsolutionofCurcuminforUVVisibleSpectroscopyCurcumin10mgwasaccurately weighed and transferred in a 100ml volumetric flask. Methanol was added upto the mark toobtain a concentration of 100µg/ml of Stock solution. From Stock solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7mlofsolutionswerewithdrawnanddilutedto10mlwithmethanoltoobtainconcentrationsof1,2,3,4,5,6, 7µg/ml, respectively [8, 9] .

Figure-2 Change in colour for each addition

II. Results and Discussion

Qualitative Phytochemical analysis showed the positive tests for the following compounds and was confirmed by the H-1 and C-13 NMR studies .Elemental analysis showed the number of C, H and O in the total compounds. Mass predictor showed the molecular weight of individual compounds.In this CUPRAC assay Spectrophotometric studies explained the behavior of phyto compounds and their antioxidant capacities. Ethanol extract showed high antioxidant capacity because of having larger number of electro active phyto compounds in organic solvents

Our study showed that the CUPRAC standard colorimetric tests involved all the active compounds in the evaluation of the antioxidant potential and Determined the maximum wavelength by UV Visible Spectroscopy Curcumin 5 µg/ml solution wasscanned in UV spectrophotometer in the range of 200-800nm methanol was used as blank. Wavelengthcorresponding to maximum absorbance of curcumin in methanol was observed at 424nm. WavelengthcorrespondingtomaximumabsorbanceofPipernigruminmethanolwasobservedat324nm.Wavelengthcorrespondingto maximumabsorbance ofinmethanolwasobservedat298nm.

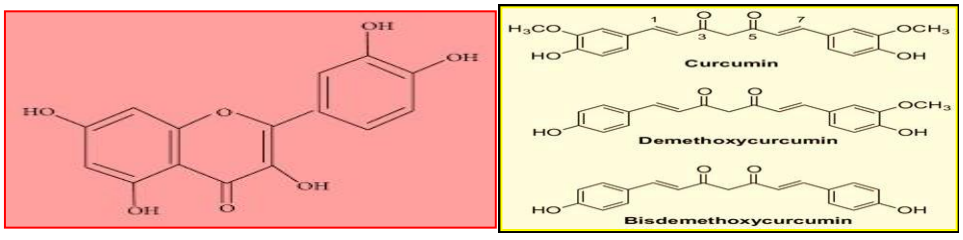


Figure-2Flavonoid

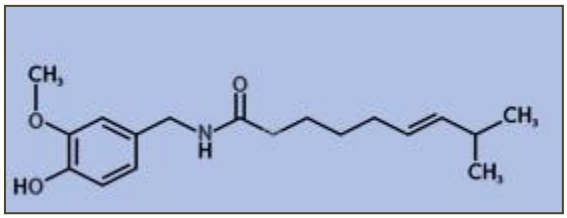


Figure-3Piperine

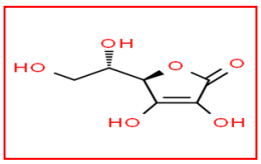
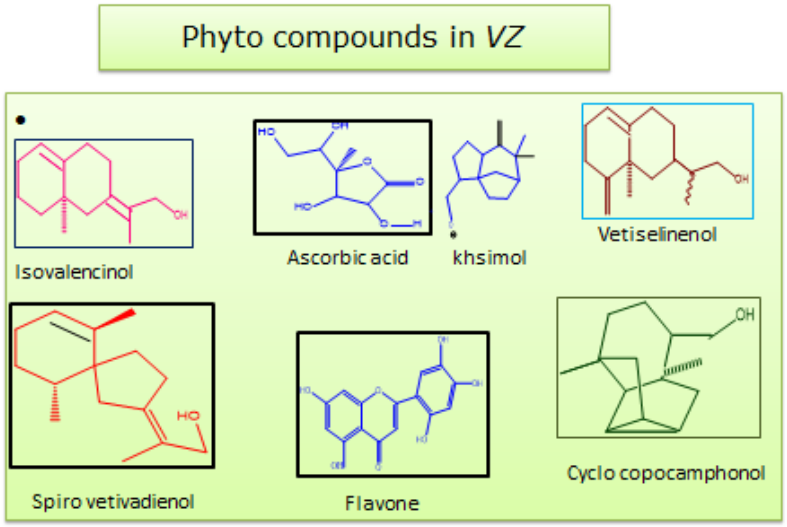


Figure-4Ascorbicacid



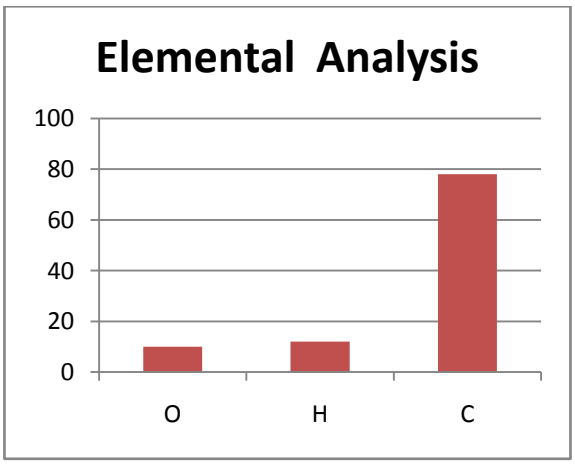


Figure-4 Curcumine

Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
H	9.14	7.38	2-pyridine, in DMSO
H	6.83	7.38	2-pyridine, in DMSO
CH	7.40	7.38	2-pyridine, in DMSO
CH	8.16	8.00	quinoline
CH	8.16	8.00	quinoline
CH	7.89	7.68	quinoline
CH	7.89	7.43	quinoline
CH	7.47	7.75	2-pyridine, in DMSO
CH	7.56	7.75	2-pyridine, in DMSO
CH3	0.9	0.86	methyl
CH3	3.72	0.86	methyl
	2.77	1 alpha -N*R	
CH3	3.72	0.86	methyl
CH3	2.53	0.86	methyl
CH3	2.53	0.86	methyl
CH3	2.53	0.86	methyl
CH3	1.89	0.86	methyl

Chemical Formula: C31H33CuN4
Exact Mass: 524.20
Molecular Weight: 525.17
m/z: 524.20 (100.0%), 526.20 (45.1%), 525.20 (35.0%), 527.20 (15.9%), 526.21 (5.6%), 528.21 (2.5%)
Elemental Analysis: C, 70.90; H, 6.33; Cu, 12.10; N, 10.67

Protocol of the C-13 NMR Prediction:

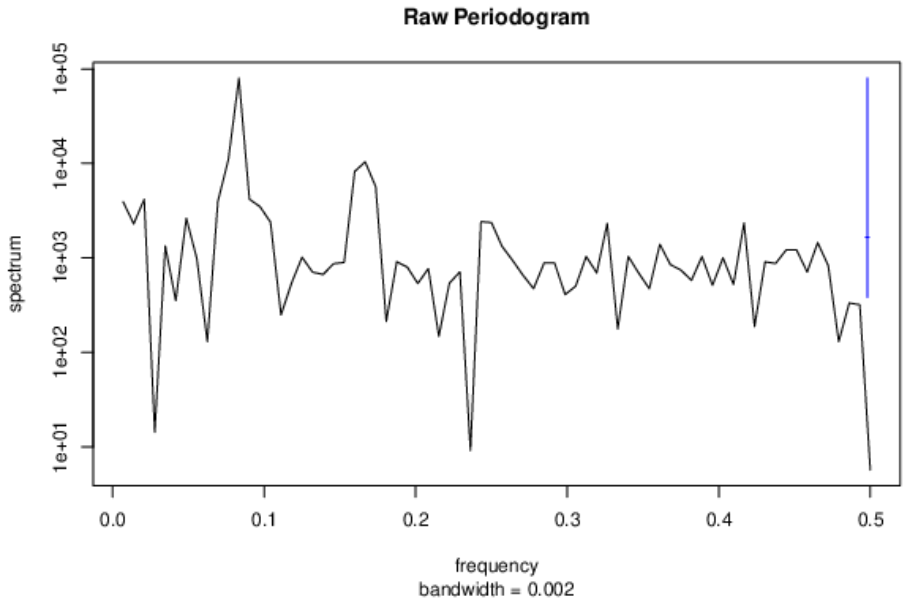
Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
C	159.8	150.0	quinoline
C	158.8	150.0	quinoline
C	135.7	148.1	quinoline
C	135.7	129.2	quinoline
C	138.6	148.1	quinoline
C	149.1	129.2	quinoline

C	127.7	128.0	quinoline
C	127.7	129.2	quinoline
CH	122.0	120.8	quinoline
CH	135.6	135.7	quinoline
CH	126.6	127.6	quinoline
CH	126.6	126.3	quinoline
CH	127.2	126.3	quinoline
CH3	4	-2.3	aliphatic
CH3	30.6	-2.3	aliphatic
CH3	30.6	-2.3	aliphatic
C	164.6	162.8	1-imine
CH3	19.8	-2.3	aliphatic
CH3	19.8	-2.3	aliphatic
CH3	24.3	-2.3	aliphatic
CH3	21.2	-2.3	aliphatic

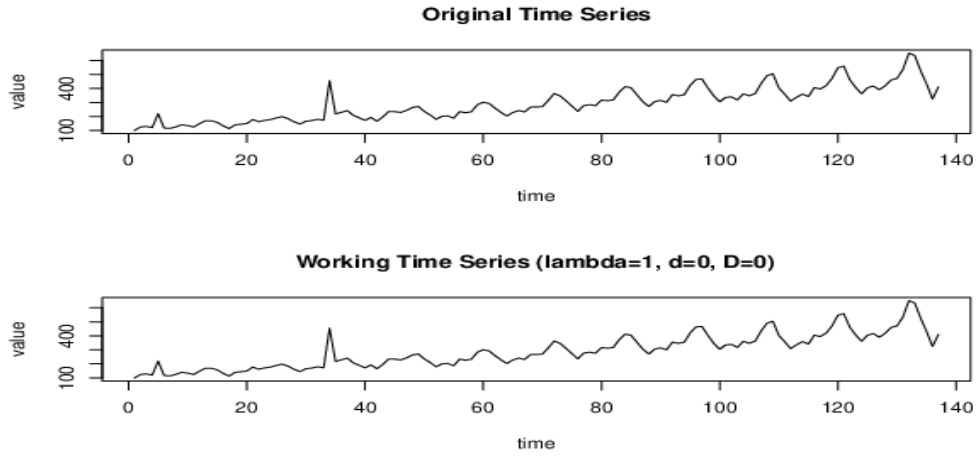
Novelty

- comparativestudyonantioxidantpotentialofCurcumin,VetiveriazizanioidesandPipernigrum
- Greenermethodtoprepareextraction–Sonication
- Identificationofphytocompoundswithreferencetoantioxidantinaqueousandorganicextracts.

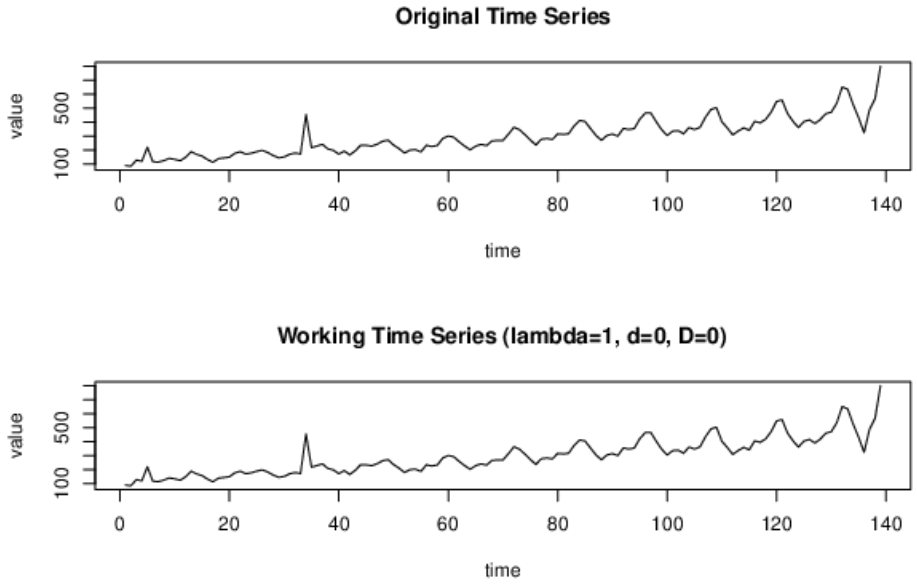
NMR studies explained the arrangements of carbon and hydrogen atoms in the complex skeleton in each addition of antioxidants and explained the potential of individual phytocompounds. It explained the Single-electron transfer (SET) oxidation-induced C–H bond functionalization usually proceeds smoothly under mild conditions due to the assistance of an oxidant, light, or electricity.
ARIMA Modeling Software explained the peaks and absorption values for all the compounds with respect to time as showed in the following figures.It emphasizedthesignificantdifferenceinchemicalcompositionamongthe*curcumin,pepper*andVetiveriazizanioides on biological activities. The concentrations of phenolic and carotenoids were determined to behigher in curcumin; however flavonoids and tannins were much more abundant in it. All the solvent extracts areendowed with potent antioxidant especially organic extract.



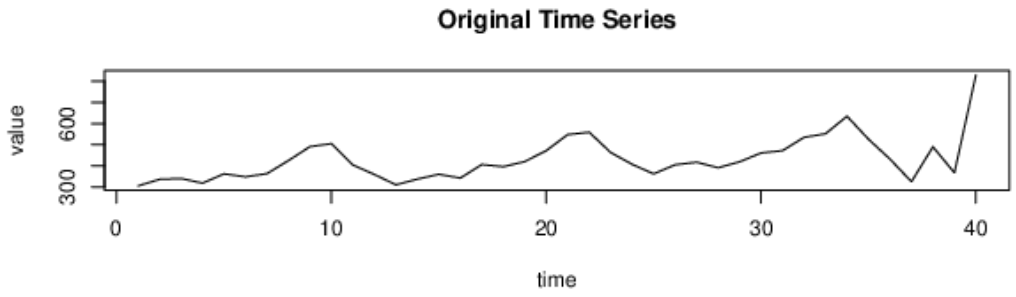
Peaks for Curcumin

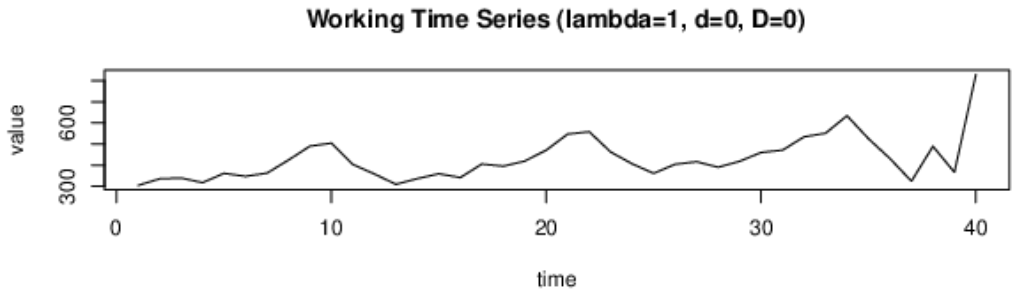


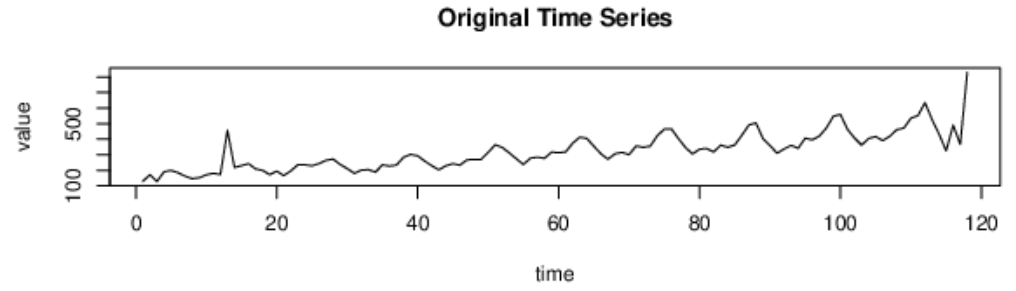
Peaks for PN

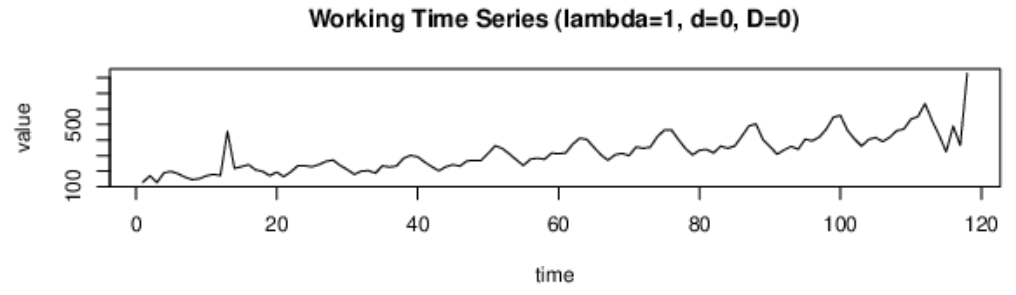


Peaks for VZ









This analysis was carried out to profile ofthe extracts, with ascorbic acid and conjugated compounds were found in a highest level. In findingsreportedthatphytocompoundscouldbenaturalsourcesofpolyphenolsflavonoids,curcumineandalkaloidsco mpounds with antioxidant properties. Antioxidant property of these extractswereidentifiedintheincreasingorderoftheplantsasaqueousextractsofVetiveriazizanioides<aqueousextracts of Piper nigrum < acetone extract of Vetiveriazizanioides< acetone extracts of Piper nigrum<Curcumin extractsfor all solvents.

III. Conclusion

Thisworkemphasizedthesignificantdifferenceinchemicalcompositionamongthe*curcumin*,*pepper*andVetiveriaziza nioides on biological activities. The concentrations of phenolic and carotenoids were determined to behigher in curcumin; however flavonoids and tannins were much more abundant in it. All the solvent extracts areendowed with potent antioxidant especially organic extract. UV Photo reactor analysis was carried out to profile ofthe extracts, with ascorbic acid and conjugated compounds were found in a highest level. In summary, findingsreportedthatphytocompoundscouldbenaturalsourcesofpolyphenolsflavonoids,curcumineandalkaloidsco mpounds with antioxidant properties. It will be interesting to draw attention to in vivo tests, to identify and purifyphenolic compounds and to confirm the beneficial quality of theseplants. Antioxidant property of these extractswereidentifiedintheincreasingorderoftheplantsasaqueousextractsofVetiveriazizanioides<aqueousextracts of Piper nigrum < acetone extract of Vetiveriazizanioides< acetone extracts of Piper nigrum<Curcumin Extractsfor all solvents . To develop these significant sources of natural antioxidants, further extraction withdifferentorganicsolventsandcharacterizationofthephenolic compositionisneeded

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