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Research Article

EXTRACTION, METHOD DEVELOPMENT, AND ANALYTICAL VALIDATION OF CRUDE AND MARKETED DRUGS

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ABSTRACT

The present study was aimed at developing "Extraction, method development and Analytical validation of crude and marketed drugs by using UV Spectroscopy method. In order to effect analysis of the component peaks, different suitable compound is tested by using different polar and non-polar solvents like Dimethyl Sulfoxide, Benzene, Acetone, Ethyl acetate, Ethanol, Methanol, Diethyl ether, distilled water etc. Extractions of different compounds are carried out using different solvents like Ethanol for Sage, Ethyl acetate for basil, Distilled water for Garlic. Extraction is done by using Soxhelet apparatus. For this process different quantities components of are taken like Sage Powder (96,8g), Basil powder (15.7g), Garlic (90g). In this process the powders are placed in a Soxhelet apparatus. 200ml of each solvent is taken for the extraction of each plant component. Before the extraction process, the plant parts are taken in required quantities like Sage leaves (200g), Basil leaves (37.2g), Garlic (250g) are dried under sunlight in shade. Along with these plant products, marketed drugs like Lopinavir and Efavirenz are taken in the quantity of 20 tablets each. After that extracted plant products aremade into dilutions and analyzed by using UV-spectrophotometer. The Sage is analyzed between 370nm-590nm; the λ max of the Sage is 580nm. The Basil is analyzed between 385nm-715nm; there are 2 λ max for Basil—Blue color obtained at 435nm and Red light obtained at 665nm. The Garlic is analyzed between 200nm-400nm; the λ max is 237nm. The Lopinavir is analyzed between 200nm-350nm; the λ max is obtained at 284nm. The Efavirenz is analyzed between 200nm-300nm; the λ max is obtained at 248nm. All these values are noted and graph is drawn.

Keywords: Efavirenz, Lopinavir, UV spectroscopy, Soxhelet apparetus, TLC, morphological characters, active constituents.

INTRODUCTION

Drug Profile:

The present study was aimed at developing "Extraction method development and Analytical validation of crude and marketed drugs". In this we took three crude drug (Sage, Basil, Garlic) and two pure drugs (Lopinavir and Efavirenz). We took the plants like Sage (Salvia Officinalis), Basil (*Ocimum basilium* L.), Garlic (*Allium Sativum*) from families like Lamiaceae (Sage, Basil) and Amaryllidaceae (Garlic) with Clade Tracheophytes, Angiosperms. Sage and Basil leaves are collected whereas for Garlic buds from roots are collected. The plants we collected are having different Anatomical and Morphological properties. Considering the uses of the taken plants and crude drugs, Sage having the uses like anti-HIV activity (Safficinolide and sage one), Culinary

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uses like used as flavor in different food items, can inhibit combat HSV-1, Indiana vesiculovirs- which infect farm animals like horses, cows and pigs. Sage is also used externally for hair, skin and nails, treat sore throat, cancer sore, gum disease, treat fungal infections, a table spoon of sage has 43% of the daily recommended serving of Vitamin K, Excellent source of Fiber, Vitamins (N, B, C, E), Folate, Calcium, Iron, Magnesium and Manganese, Thiamin, Copper. Basil having the uses like Apigenin and Urosolic acid compounds in basil have demon striated effects against Herpes viruses, Hepatitis B and Entero viruses, different parts of basil like leaves, seeds are used as flovour in many food preparations, used in oil preparations, basil is also used in folk medicine, Insecticide and insect repellent, Nematicide, Bacterial and fungal inhibition. Garlic having the uses like effectiveness in staving of influenza, viral pneumonia and rhinovirus- which causes the common cold, to treat Cardiovascular problems, to decrease Cancer, used as an adhesive in mending glass and porcelain, nematicide and insecticide, used in folk medicine, for protection or white magic, a powerful ward against demons, werewolves, and vampires. Lopinavir plays a critical role in many aspects of the HIV viral lifecycle as an anti-viral agent. Efavirenz is used as an Anti-HIV agent by altering the DNA/RNA of virus, also used to treat other antiretrovirals, Efavirenz in combination with tenofovir/emtricitabine (Truvada) as one of the preferred NNRTI-based regimens in adults and adolescents and children is also used. Efavirenz is also used in combination with other antiretroviral agents as part of an expanded post-exposure prophylaxis regimen to reduce the risk of HIV infection in people exposed to a significant risk (e.g. need lestick injuries, certain types of unprotected sex, etc).

ANTI-VIRAL DRUGS:

Anti-viral drugs are class of medication to treat viral infections. Most of anti-viral drugs are target specific viruses, while a broad-spectrum antiviral is effective against wide range of viruses. These inhibit the development of viruses.

ANTI-VIRAL TARGETTING:

The antiviral drug design is to identify viral proteins or parts of proteins that can be disabled. These targets should also common across many strains of virus even among different species of virus in the same family. So, single drug will have broad effectiveness.

UV-SPECTROSCOPY:

In the region of electro-magnetic spectrum molecules containing non-bonding electrons (n-electrons) can absorb energy in the form of Ultraviolet or Visible light to excite these electrons to higher anti-bonding molecular orbitals. The earlier excitation of electrons the longer the wavelength of light it can absorb.

CHROMATOGRAPHY:

It is a physical process where the components (solutes) of a sample mixture are separated as result of their differential distribution between stationary and mobile phases. In Greek chroma meaning 'color' and graphein meaning 'writing'. Tswet, Russian botanist (referred to as father of chromatography) is credited for the development of chromatography. The Mobile Phase usually refer to the mixture of the substances to be separated dissolved in a liquid or a gas. The Stationary Phase is a porous solid matrix through which the sample contained in the mobile phase percolates. The retention time or volume is when a solute exits the injector and passes through the column and the detector.

Chromatogram-

A graphical representation of detector response, concentration of analyte in the effluent, or other quantity used as a measure of effluent concentration.

Classification- These methods can be classified in three different ways: -

a. Based on shape of chromatographic beds. E.g. - Planar (Paper and TLC) and Column (Gas and Liquid) Chromatography.
b. Based on physical state of mobile and stationary phase. E.g. – Gas and Liquid Chromatography.
c. Based on mechanism of separation. E.g. – Ion exchange chromatography, partition, affinity and adsorption chromatography.

VALIDATION PARAMETERS:

1. **Accuracy:**
   It is defined as the degree of closeness of experimental value to the true value.
2. **Precision:**
   It is defined as the degree of agreement among individual. This results when method is applied to homogenous sample under given conditions.
   Intraday- The sample was analyzed for three times in a day (Morning, Afternoon and Evening).
   Interday- The sample was analyzed for three different days in different days.
3. **Limit of Detection:**
   It is defined as the lower amount of analyte on a sample which can be detected but not necessarily quantified as an exact value.
4. **Limit of Quantification:**
   It is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
5. **Range:**
   It is defined as the lowest and highest value of analyte in the sample.

6. **Robustness:**
   It is defined as an analytical procedure in measure of its capacity to remain unaffected by small but deliberate variation in method parameters.
7. **Linearity:**
   It is defined as the elect test results are directly proportional to concentrate of analyte present in the sample.

MATERIALS:

Materials Required for Extraction Process:

The materials required for the extraction process are, the required parts of plants (Leaves of Sage and Basil, Buds of Garlic) and the marketed drugs (Lopinavir and Efavirenz), mixer or grinder, sieves, weighing balance, butter paper, spatula, beaker, soxhelet apparatus, condenser, cotton plug, card board sheet, round bottom flask, burette stand, Bunsen burner, tripod stand, required solvent (Ethanol, Methanol, Distilled water, Dimethyl Sulphoxide, Ethyl acetate), water pipes, refrigerator, water for washing, Aluminium sheet, cotton, rubber.

Materials Required for Paper and thin Layer Chromatography:

Extracted materials (Sage, Basil, Garlic), beakers, required solvents (Butan-1-ol: Glacial acetic acid: Distilled water) in required ratio (4:1:5), chromatogram (whattman filter paper), capillary tube, lid, thread, pencil, chromatography tank, glass slide, silicon.

Materials Required for UV-Spectroscopy and Validation:

UV-Spectrophotometer, cuvettes, test tubes, beakers, measuring cylinders, required solvents (Ethanol, Methanol, distilled water, Dimethyl sulphoxide, Ethyl acetate), extracted materials (Sage, Basil, Garlic) and marketed drugs (Lopinavir, Efaverinz), water for washing, tissue paper.

METHODS:

Extraction Method:

Before doing extraction, the leaves of Sage, Basil and Buds of Garlic are dried in sun under shade. After drying they are crushed using mixer and sieved with 70 mesh sieves. The powdered and sieved powder of plant materials were taken in a required quantity and packed in a card board sheet. Cotton is placed in soxhelet apparatus and packed powders are placed in apparatus which is fixed to the condenser with water pipes and one of the pipes is fixed to the water tap and another pipe is leaved into sink. Required solvents are placed in a round bottom flask for each plant material and fixed to the soxhelet apparatus using cotton. This is placed on tripod stand by using burette stand and heated by using Bunsen burner. This heating is continued for 6-8 hours. After that the extracted material is filtered by using whattman filter paper and funnel and placed in a refrigerator.

Chromatography Method:

Whattman filter paper is taken in a required length and width. Line is drawn on 2cm above from the bottom of the paper. The points are drawn by using the pencil and the spots of the extracted materials are placed on the points by using the capillary tube. The spotted paper is placed in a beaker filled with the solvents and leaved for 45 mins or until $3/4^{th}$ of the paper is get wet. The paper should be placed in the way that the spots should not touch the solvents. After that the separated compound are identified.

UV-SPECTROSCOPY METHOD:

The extracted materials and marketed drugs are analyzed using spectroscopic methods. These drugs are diluted into dilutions (2ml, 4ml, 6ml, 8ml and 10 ml by using the suitable solvents. From that the middle dilution is taken and filled in a cuvette and analyzed by respected solvent as blank and placed in the spectrophotometer. In this process the nanometers are settled in the spectrophotometer based on the solvent analyzing. Then the results are noted especially λ_{max} values are noted.

VALIDATION METHODS:

Linearity:

The linearity was performed by preparing different concentrations (6.25, 12.5, 25, 50, and 100µg/ml) from the stock solutions of 10µg/ml of extracted materials and marked drugs which are to be validate. The solution of 20µl was injected into columns three times. Linearity of solutions are noted with the correlation coefficient.

Accuracy:

The accuracy was studied by preparing standard solution of different concentrations (10, 35, 55 µg/ml) and injected to check the % recovery. The percent recovery of the drug was found in the range of 98.8 to 102.0% respectively for all concentrations.

Precision:

The precision was checked by injecting a solution of 80µg/ml for six times in same days, different days, and in a different time interval on the same day. The % RSD was found to be less than 3 %, which showed good precision.

Specificity:

Specificity was performed to determine the retention time of each drug in a mixture and in the sample. The retention time of standard drugs individually was determined, and it was found to be different for different drugs.

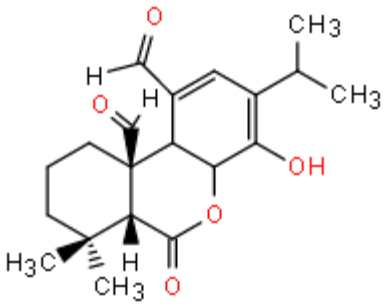
Limit of Detection (LOD):

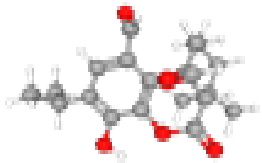
A blank resolution is injected and peak to peak quantitative noise relation we have to calculate from blank chromatograms. Then, calculate the concentration at the signal to quantitative noise relation is concering 3:1.

LOD can be expressed as LOD= 3.3SD/S
Where, SD= Standard deviation of response
S= Slope of calibration curve

DRUG PROFILE:

SAFFICINOLIDE:

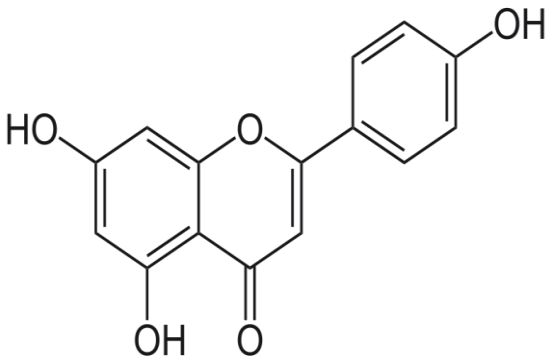




Safficinolide is a hydrocoumarin.

Property Name	**Property Value**
Molecular Formula	: C20H24O5
IUPAC Name	:4-hydroxy-7,7-dimethyl-6-oxo-3-propan-2-yl-6a, 8,9,10-tetrahydrobenzo[c]chromene-1,10a-dicarbaldehyde
Molecular Weight	: 344.4g
XLogP3-AA	: 3.3
Hydrogen Bond Donor Count	: 1
Hydrogen Bond Acceptor Count	: 5
Rotatable Bond Count	: 3
Exact Mass	: 344.16237386
Monoisotopic Mass	: 344.16237386
Topological Polar Surface Area	: 80.7 $Å^2$
Heavy Atom Count	: 25
Formal Charge	: 0
Complexity	: 568
Isotope Atom Count	: 0
Defined Atom Stereocenter Count	: 0
Undefined Bond Stereocenter Count	: 0
Covalently-Bonded Unit Count	: 1
Compound Is Canonicalized	: Yes
Physical Description	: Solid
Melting Point	: 223-224°C
Solubility	: 92.26 mg/L @ 25°C (est)
Cellular Locations	: Extracellular, Membrane

APIGENIN:



Property Name:	**Property Value**
Molecular Formula	: $C_{15}H_{10}O_5$
IUPAC Name	: 5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one
Molecular Weight	: 270.24
XLogP3	: 1.7
Hydrogen Bond Donor Count	: 3
Hydrogen Bond Acceptor	: 5
Rotatable Bond Count	: 1
Exact Mass	: 270.05282342
Monoisotropic Mass	: 270.05282342
Topological Polar Surface Area	: 87 $Å^2$
Heavy Atom Count: 20	
Formal Charge	: 0
Complexity	: 411
Isotope Atom Count	: 0
Defined Atom Stereocenter Count	: 0
Undefined Atom Stereocenter Count: 0	
Defined Bond Stereocenter Count	: 0
Undefined Bond StereocenterCount : 0	
Covalently-Bonded Unit Count	: 1
Compound Is Canonicalized	: Yes
Physical Description	: Solid
Color/Form	: Yellow needles from aqueous pyridine
Boiling Point	: 555.5℃ @ 760.00 mm Hg (est)
Melting Point	:347.5℃ (345-350℃)
Solubility	: Soluble in ethanol, pyridine, concentrated sulfuric acid, very soluble in dilute alkalies, moderately soluble in hot alcohol, soluble in dilute KOH with intense yellow color, in water- 183 mg/L @ 25℃ (est).
Vapor Pressure	:1.01X10-10 mm Hg at 25℃ (est)
LogP	: 3.02 (LogP) (log Kow)
Henry's Law Constant	: 5.12X10-17 atm-cu m/mol at 25℃ (est)
Decomposition	: When heated to decomposition it emits acrid smoke and irritating fumes.
Dissociation Constant	: pKa1 = 7.12 (phenol); pKa2 = 8.10 (phenol)
Collision Cross Section	: 156$Å^2$ [M+H]- [CCS Type: TW, Method: calibrated with polyalanine and drug standards].
Hydroxyl radical reaction rate constant: 2.31X10-10 cu cm/molec-sec at 25℃ (est).	

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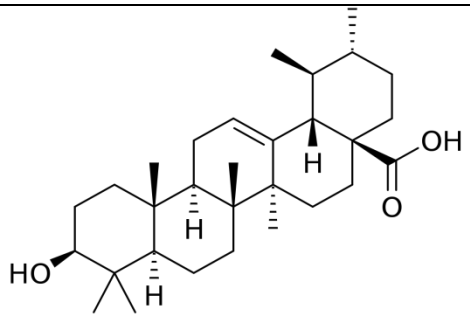

Property Name: Property Value

Molecular Formula : $C_{30}H_{48}O_3$
IUPAC Name : (1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid.
Molecular Weight : 456.7
XLogP3-AA : 7.3
Hydrogen Bond Donor Count : 2
Hydrogen Bond Acceptor Count : 3
Rotatable Bond Count : 1
Exact Mass : 456.36034539
Monoisotopic Mass : 456.36034539
Topological Polar Surface Area : 57.5$Å^2$
Heavy Atom Count : 33
Formal Change : 0
Complexity : 874
Isotope Atom Count : 0
Defined Atom StereocenterCount : 10
Undefined Atom Stereocenter Count: 0
Defined Bond StereocenterCount : 0
Undefined Bond Stereocenter Count: 0
Covalently-Bonded Unit Count : 1
Compound Is Canonicalized : Yes
Physical Description : Solid
Color/Form : Platelets from alcohol, Large, lustrous prisms from absolute alcohol, fine hail-like needles from dilute alcohol.
Melting Point : 284°C, 246-247°C
Solubility : One part dissolves in 88 parts methanol, 178 alcohol, (35 boiling alcohol), 140 ether, 388 chloroform, 1675 carbon disulfide. Moderately soluble in acetone. Soluble in hot glacial acetic acid and in 2% alcoholic NaOH. Insoluble in petroleum ether. In water, 1.02X10-4 mg/L at 25°C (est), 182.2 mg/L @ 25°C (est)
Vapor Pressure : 3.49X10-14 mm Hg at 25°C (est)
LogP : log Kow = 7.92 (est)
Henry's Law Constant : 9.66X10-10 atm-cu m/mol at 25°C (est)
Optical Rotation : +67.5 deg at 21°C/D (c = 1 in N alcohol KOH)
289-290°C - +62.3 DEG at 25°C/D (c=1.15 in chloroform
171°C - +58 deg at 20°C/D (c=1.2 in pyridine)
Hydroxyl radical reaction rate constant : 1.27X10-10 cu cm/molec-sec at 25°C (est)
Ozone radical reaction rate constant : 7.39X10-17 cu cm/molec-sec at 25°C (est)

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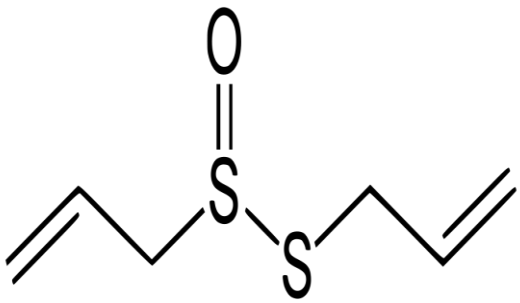

Property Name:

Molecular Formula	: $C_6H_{10}OS_2$
IUPAC Name	: 3-propo-2-enylsulfinylsulfanylprop-1-ene
Molecular Weight	: 162.3
XLogP3-AA	: 1.3
Hydrogen Bond Donor Count	: 0
Hydrogen Bond Acceptor Count	: 3
Rotatable Bond Count	: 5
Exact Mass	: 162.01730729
Monoisotropic Mass	: 162.01730729
Topological Polar Surface Area	: 61.6$Å^2$
Heavy Atom Count	: 9
Formal Charge	: 0
Complexity	: 120
Isotope Atom Count	: 0
Defined Atom StereocenterCount	:0
Undefined Atom Stereocenter Count	: 1
Defined Bond Stereocenter Count	: 0
Undefined Bond Stereocenter Count	: 0
Covalently-Bonded Unit Count	: 1
Compound Is Canonicalized	: Yes
Physical Description	: Solid
Boiling Point	: 248.60℃ @760.00 mm Hg (est)
Melting Point	: 25℃
Solubility	: 24 mg/mL at 10℃
LogP	: 1.133 (est)

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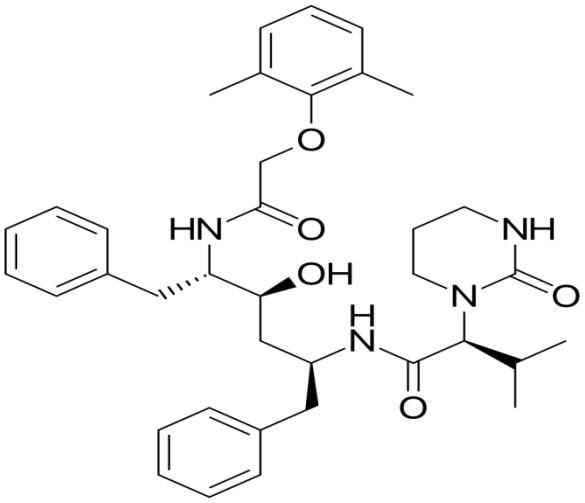
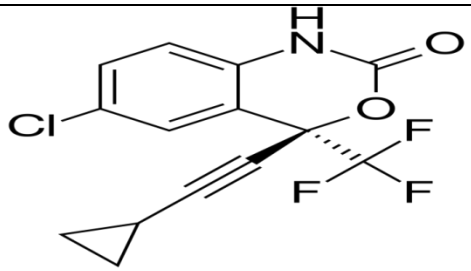


Property Name	**Property Value**
Molecular Formula	: $C_{37}H_{48}N_4O_5$
IUPAC Name	: (2S)-N-[(2S,4S,5S)-5-[[2-2(2,6-dimethylphenoxy)acetyl]amino]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide
Molecular Weight	: 628.8
XLogP3-AA	: 5.9
Hydrogen Bond Donor Count	: 4
Hydrogen Bond Acceptor Count	: 5
Rotatable Bond Count	: 15
Exact Mass	: 628.36247064
Monoisotropic Mass	: 628.36247064
Topological Polar Surface Area	: 120Å^2
Heavy Atom Count	: 46
Format Charge	: 0
Complexity	: 940
Isotope Atom Count	: 0
Defined Atom Stereocenter Count	: 4
Undefined Atom Stereocenter Count	: 0
Defined Bond Stereocenter Count	: 0
Undefined Bond StereocenterCount	: 0
Covalently-Bonded Unit Count	: 1
Compound Is Canonicalized	: Yes

EFAVIRENZ:



Property Name

Molecular Formula : $C_{14}H_9ClF_3NO_2$
IUPAC Name : (4S)-6-chloro-4-(2-cyclopropylethyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one
Molecular Weight : 315.67
XLogP3-AA : 4
Hydrogen Bond Donar Count : 1
Hydrogen Bond Acceptor Count : 5
Rotatable Bond Count : 1
Exact Mass : 315.0273907
Monoisotopic Mass : 315.0273907
Topological Polar Surface Area : 38.3Å^2
Heavy Atom Count : 21
Formal Charge : 0
Complexity : 519
Isotope Atom Count : 0
Defined Atom Stereocenter Count : 1
Undefined Atom Stereocenter Count: 0
Defined Bond Stereocenter Count : 0
Undefined Bond Stereocenter Count: 0
Covalently-Bonded Unit Count : 1
Compound Is Canoncilized : Yes
Physical Description : Solid
Color/Form : Crystals from toluene:heptanes, White to slightly pink crystalline powder.
Melting Point :139-141°C
Solubility : In water, 0.093 mg/L at 25°C (est), Practically insoluble in water (less than 10 mg/L) 8.55e-03 g/L.
Vapour Pressure : 3.8X10-7 mm Hg at 25°C (est)
LogP : 4.6, log Kow = 4.7 at 25°C (est)
Henry's Law Constant : 7X10-9 atm-cu m/mol at 25°C (est)
Dissociation Constants :pKa = 10.2, 12.52 (amine) (est)
Hydroxyl radical reaction rate constant: 4.2X10-11 cu cm/molec-sec at 25°C (est)

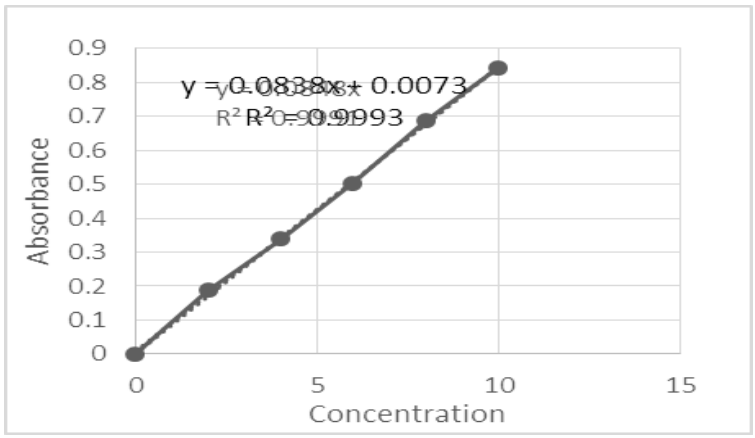
RESULTS:

Sl. No.	Validation Parameters	Extracted Herbal Drugs			Marketed Drugs	
		Sage	**Basil**	**Garlic**	**Efavirenz**	**Lopinavir**
1	λ_{max}	280nm 580nm	435nm for Blue 665nm for Red	237nm	284nm	248nm
2	Linearity	63.140	36.760	36.043	41.395	57.921
3	Accuracy	80%- 57.46 100%- 30.88 120%- 42.36	80%- 49.98 100%- 30.88 120%- 40.59	80%- 50.79 100%- 35.74 120%- 42.22	80%- 91.82 100%- 35.40 120%- 34.58	80%- 29.98 100%- 32.50 120%- 33.75
4	Precision Intraday Interday	19.89 13.18	21.42 14.05	14.92 15.04	88.149 51.19	17.98 20.19
5	Limit of Quantitation	0.039	0.0286	0.0408	0.0665	0.0408
6	Limit of Detection	0.188	0.086	0.123	0.200	0.123
7	Robustness	580- 78.5%	435- 76.5% 665- 77.4%	253- 72.3%	245-84.01%	248- 68% 253-79%
8	Ruggedness	Set 1: 79% Set 2: 81%	Set 1: 85% Set 2:88%	Set 1: 89% Set 2: 92%	Set 1: 80% Set 2: 86%	Set1: 85% Set2: 89%

Linearity:

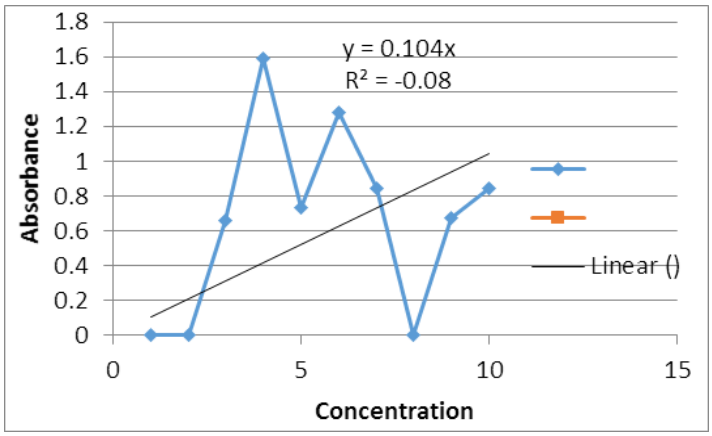
S.No.	Concentration	Absorbance
1	**0**	**0**
2	**2**	**0.1882**
3	**4**	**0.3394**

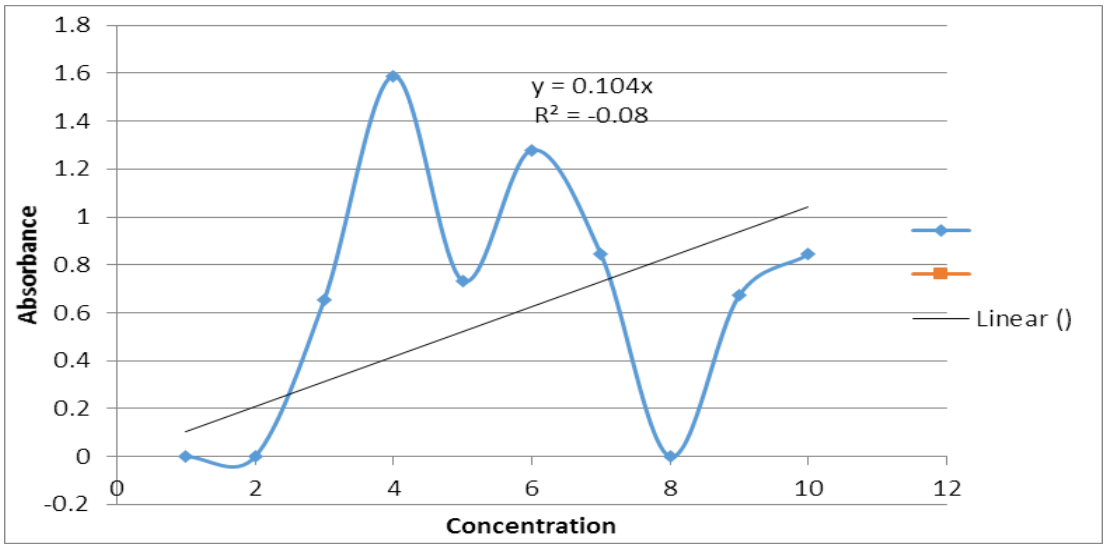
4	**6**	**0.5034**
5	**8**	**0.6858**
6	**10**	**0.8424**



Accuracy:

S.No.	Concentration	Absorbance
	0	**0**
	80	**0.6556** **1.5892** **0.7332**
	100	**1.2796** **0.844** **1.5776**
	120	**0.6722** **0.8434** **1.3464**

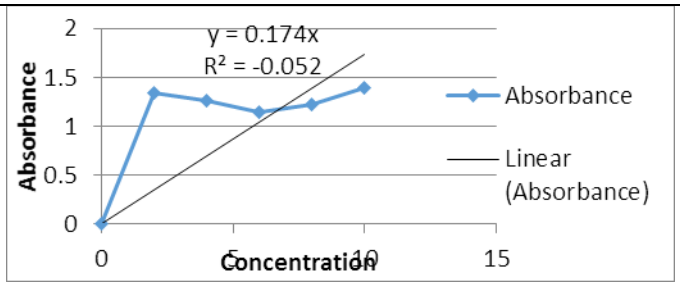


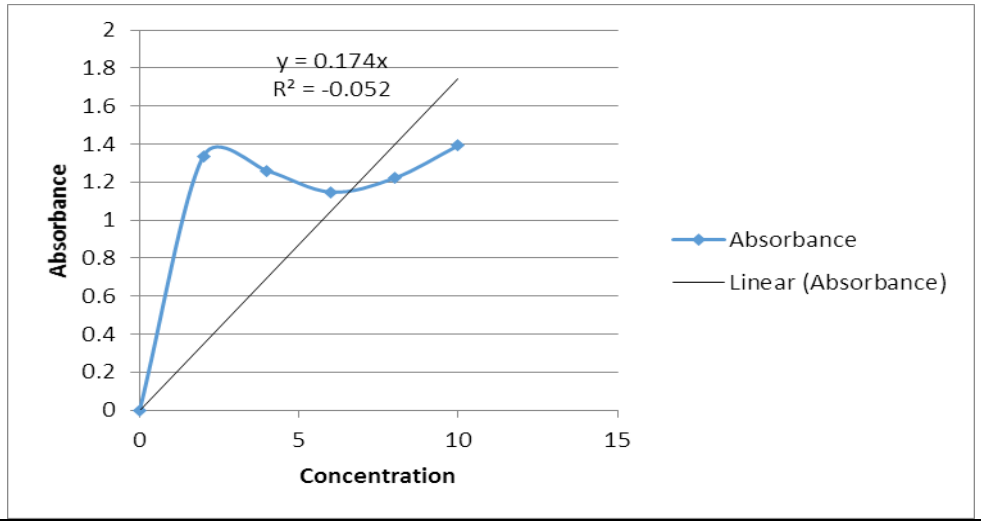


Interday-1:

S.No.	Concentration	Absorbance
1	**0**	**0**
2	**2**	**1.337**
3	**4**	**1.2596**
4	**6**	**1.1476**
5	**8**	**1.221**

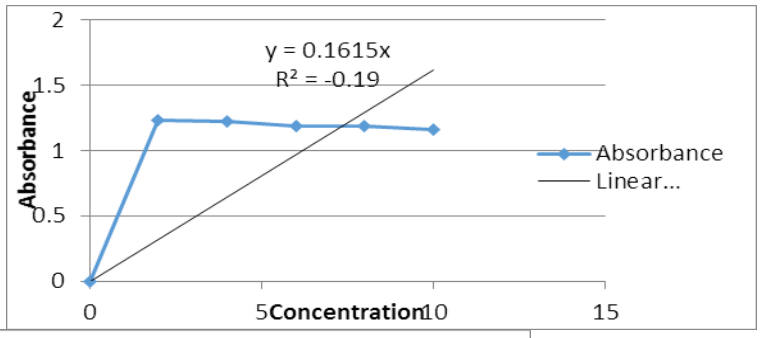
6	**10**	**1.3924**

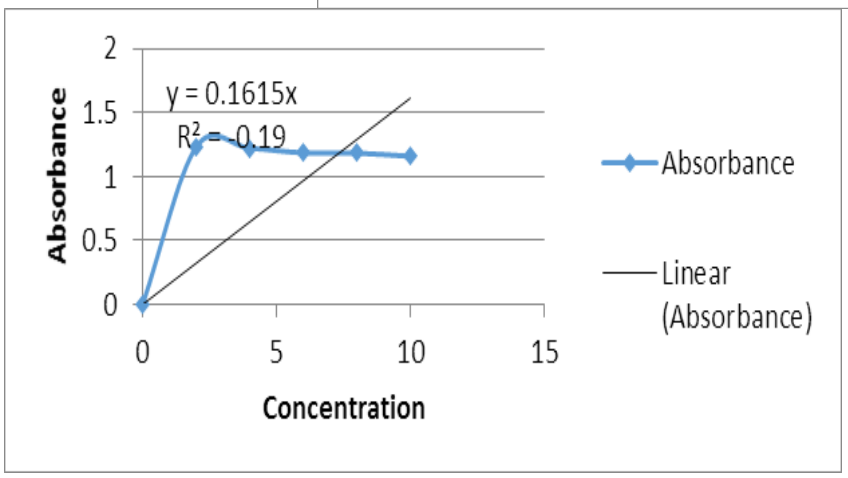




Interday-2:

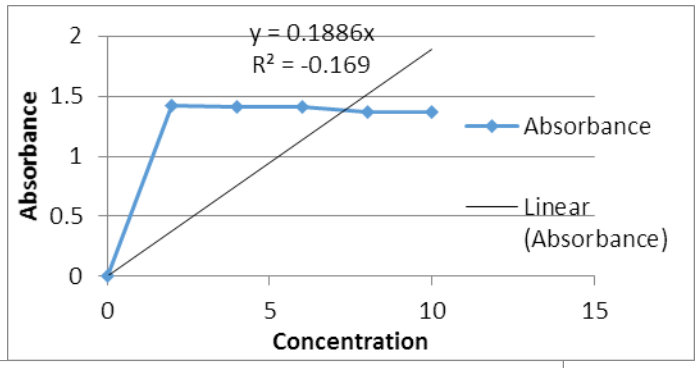
S.No.	Concentration	Absorbance
1	**0**	**0**
2	**2**	**1.2318**
3	**4**	**1.222**
4	**6**	**1.1858**
5	**8**	**1.184**
6	**10**	**1.159**

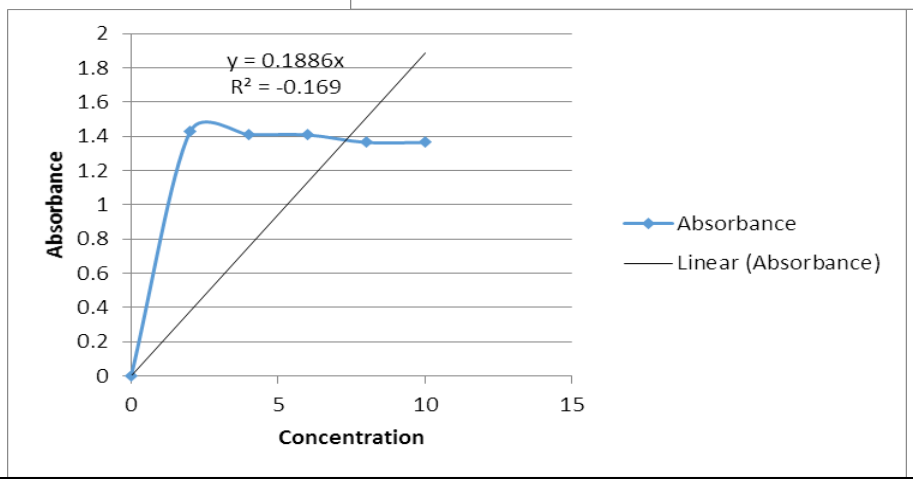




Interday-3:

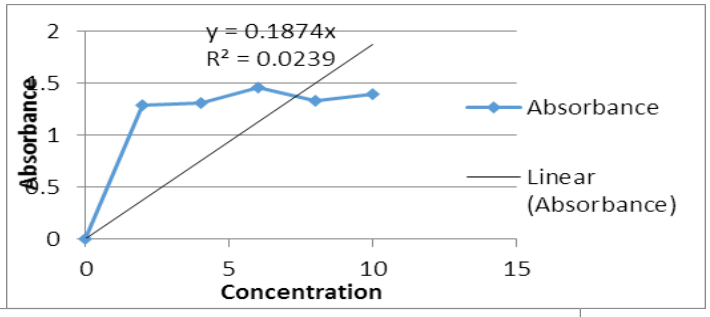
S.No.	Concentration	Absorbance
1	**0**	**0**
2	**2**	**1.4258**
3	**4**	**1.4092**
4	**6**	**1.408**
5	**8**	**1.3638**
6	**10**	**1.3644**

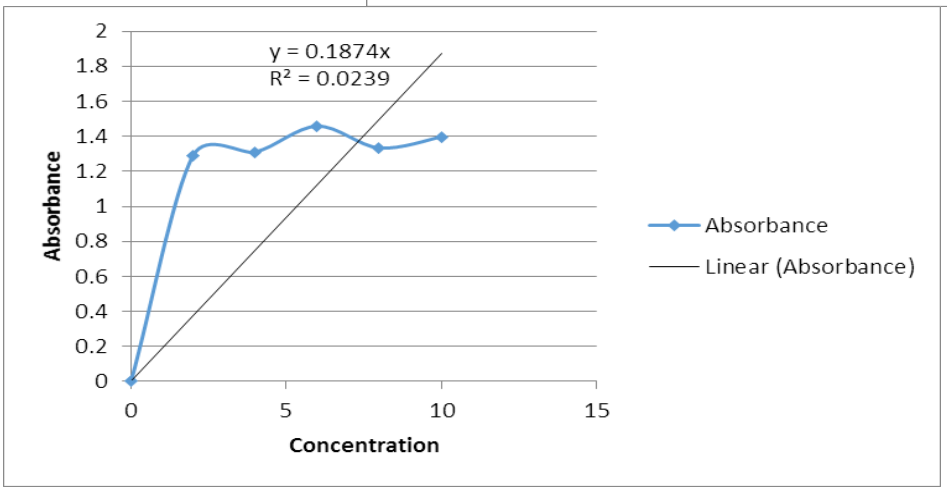




Intraday-1:

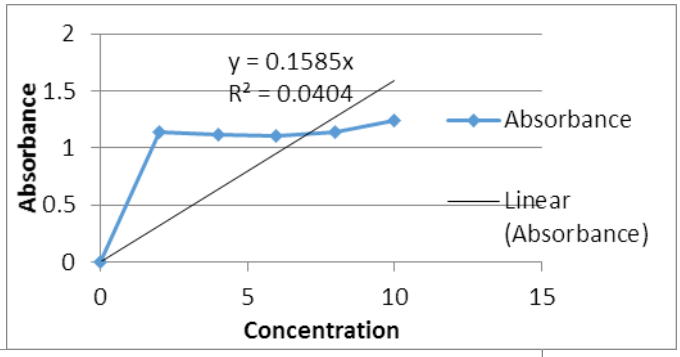
S.No.	Concentration	Absorbance
1	**0**	**0**
2	**2**	**1.2906**
3	**4**	**1.3104**
4	**6**	**1.4586**
5	**8**	**1.3338**
6	**10**	**1.3976**

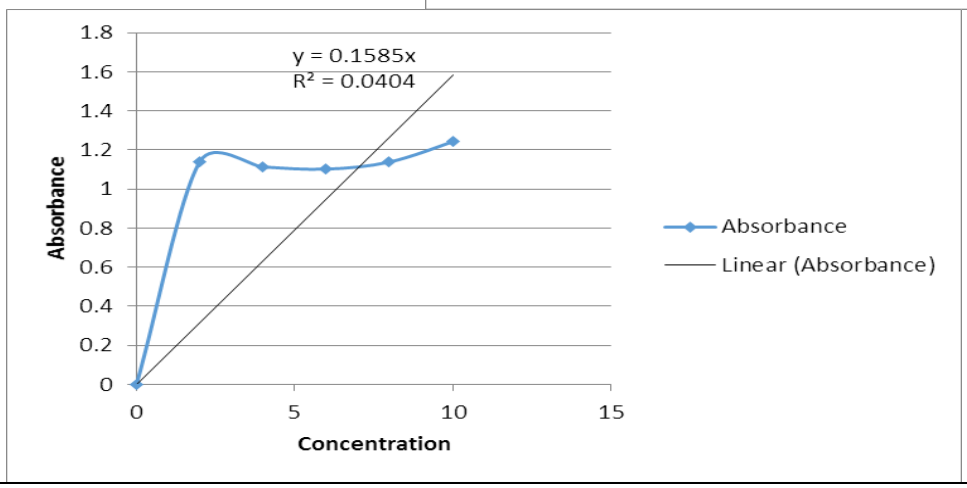




Intraday-2:

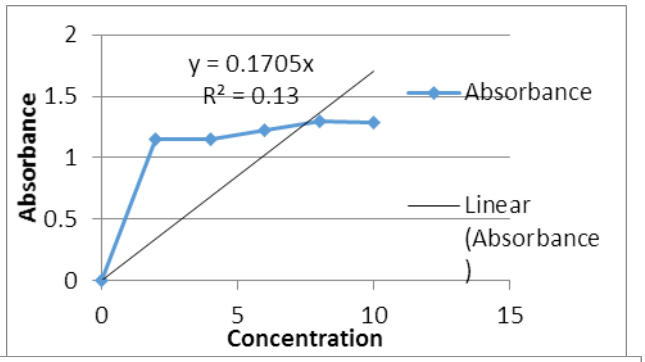
S.No.	Concentration	Absorbance
1	0	0
2	2	1.1398
3	4	1.1132
4	6	1.102
5	8	1.1382
6	10	1.242

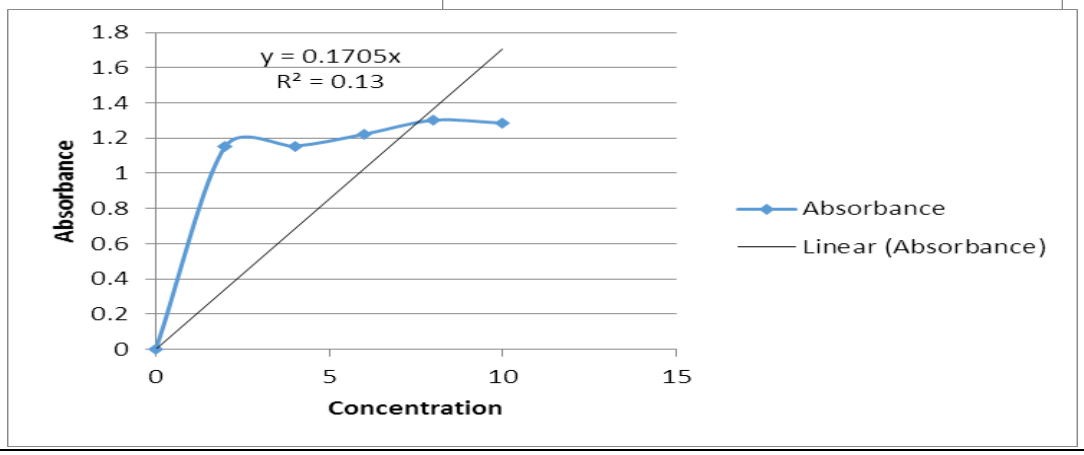




Intraday-3:

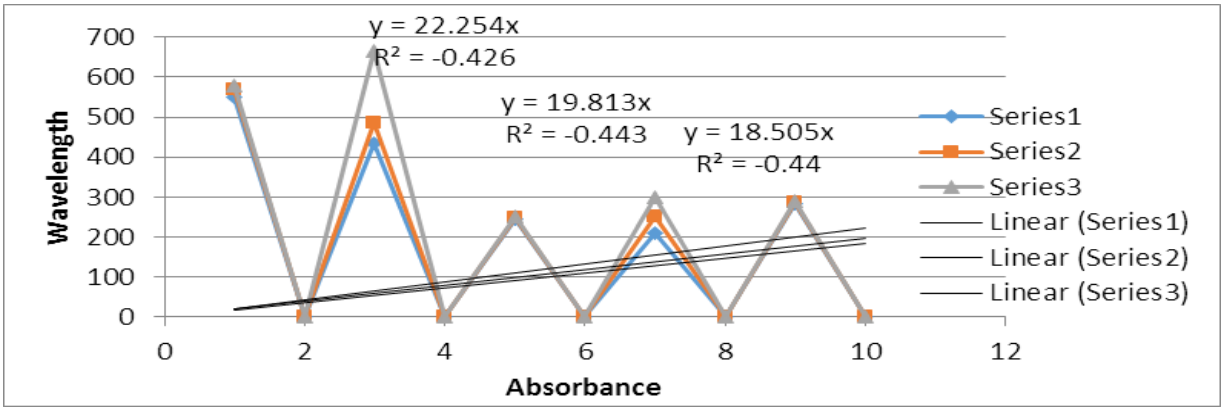
S.No.	Concentration	Absorbance
1	0	0
2	2	1.1544
3	4	1.1544
4	6	1.222
5	8	1.3016
6	10	1.2852

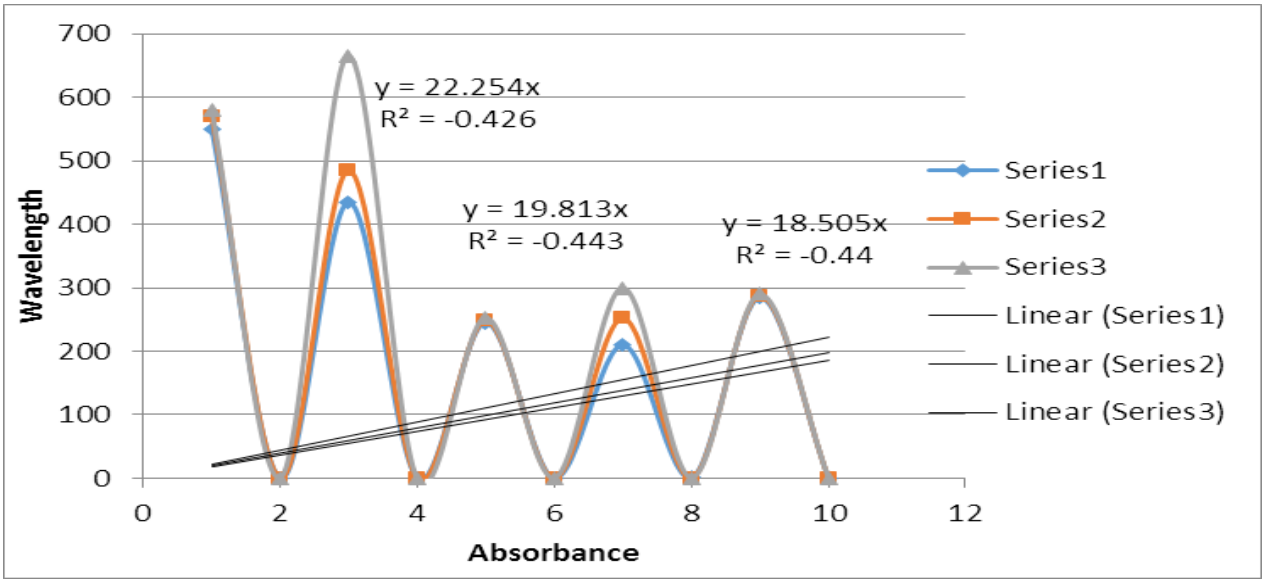




Robustness:

SAGE		**BASIL**		**GARLIC**		**LOPINAVIR**		**EFAVIRENZ**	
Wave length	Absorbance	Wave length	Absorbance	Wave length	Absorbance	Wave length	Absorbance	Wave length	Absorbance





Limit of Quantitation (LOQ):

It is characterized by the least quantity of an analyte that can be quantified with exactness and precision.

LOQ can be communicated as LOQ= 10SD/S

Some usual techniques, methods for the assessment of LOD and LOQ are as follows:

Visual inspection

Signal to noise ratio,

Standard deviation of the bank, and

Regression line at low concentration.

CONCLUSION:

The consideration of all the results we finally concluded that the extracted crude (Sage, Basil, Garlic) drugs have some antiviral activity. However, we didn't find the significant value (Percentage) of it. If anyone has keen interest to make the project to forward, research it. It would be better if you do within animal house on animals.

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