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To Study Proximate Analysis & Biological Evalution Of Triphala Guggulu Formulation

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Abstract: Triphala guggulu is one of ayurvedic medicinal preparation is Poly herbal formulation made up of mixtures of five drugs and these drugs contain variety of phytoconstituents. Many scientists agree that if a herbal medicinal product contains several herbal drugs and if it is not possible to perform a quantitative determination of each active substance, the determination may be carried out jointly for several similar active substance. Triphala guggulu may be considered as 'polyherbal medicinal product' made up of multiple numbers of constituents and hence in present investigation major important constitutents were estimated in totality not for any individual markers. The total phenolic content was determined by Folin & ciocalteu's phenol reagent method by UV-spectroscopic method and total flavanoid content were determined by UV-spectroscopic method by using aluminium chloride methods. Herbal antioxidant like triphla guggulu have exhibit significant immunomodulator activity, so that we checked antioxidant activity of triphala guggulu with respective of standard sample of ascorbic acid & we checked the antibacterial activity by cup &plate method

Key words: Antioxidant activity, antibacterial, Total phenolic, Total flavonoid content**.**

INTRODUCTION

Triphala guggulu is one of ayurvedic medicinal preparation is Poly herbal formulation made up of mixtures of five drugs and these drugs contain variety of phytoconstituents. Many scientists agree that if a herbal medicinal product contains several herbal drugs and if it is not possible to perform a quantitative determination of each active substance, the determination may be carried out jointly for several similar active substance. Triphala guggulu may be considered as 'polyherbal medicinal product' made up of multiple numbers of constituents and hence in present investigation major important constitutents were estimated in totality not for any individual markers. The total phenolic content and total flavanoid content were determined using standard methods.

MATERIAL & METHOD:

Estimation of total phenolic content by uv spectroscopic method[1,3,4]:
Principle:-
Folin &ciocalteu's phenol reagent dose not contain phenol. Rather the reagent with react with phenols and non-phenolics reducing substance to form

chromogens that can be detected spectro photometrically. The colour development is due to the transfer of electrons at basic pH to reduce phosphomolybdic/ phosphotungstic acid complexes to form chromogens in which the metals have lower valence.

Preparation Of Methanolic Extracts Of Triphala Guggulu

(0.1gm) of methanolic extracts of triphala guggul was dissolved in 10 ml distilled water in 10 ml volumetric flasks. 1 ml of this solution diluted up to 10 ml with water. The same procedure was performed for each sample of triphala guggul and solution (100) ml of triphala guggulu extracts was prepared.

Reagents:

- Folin-ciocaltue's reagent
- 7% Na2co3 solution prepared in distilled water

Standard Solution Of Gallic Acid:

Accurately weighed gallic acid (100mg) was dissolved in distilled water and made up to 100 ml with distilled water in volumetric flask. Aliquotes of this solution were further diluted to get test solutions with concentration of 100µgm/ml to 1000µgm/ml.

Calibration curve:

A series of calibrated 10ml volumetric flasks were taken and appropriate aliquots of the 100µgm/ml-1000µgm/ml concentration of working standard solution of gallic acid were pipette out. To each flask was added distilled water 3ml, Folin-ciocaltue's reagent(250µl), sodium carbonate solution(750µl) and incubated for 8 min at the room temperature then add distilled water (950µl) .the absorbance for so formed blue colored complex was measured at absorption maxima 765 nm after 1h, against the reagent blank prepared in the similar manner without the gallic acid. The absorption maxima and beer's law limit were recorded and the data that prove the linearity and obey beer's law were noted.

The linear correlation between these concentration (x-axis) and absorbance (y-axis) was graphically presented and the slop(b), intercept(a), and correlation coefficient(r) were calculated out for linear equation(y=bx+a) by regression analysis using the method of least square.

Estimation of total phenolic content in test solutions

The appropriate aliquots from extracts of each sample of triphala guggulu were withdrawn in 10 ml volumetric flasks separately. The blue colored complex was developed in the similar manner as in calibration curve studies, replacing the gallic acid the triphala guggulu extracts and the absorbance for aliquots of each sample was noted at 765nm. The responding concentration of triphala guggulu against respective absorbance were determined as gallic acid using the calibration curve.

Estimation of total flavonoid content by UV-spectroscopic Method[6,7]

Principle:-

The flavonoid on reaction with aluminum chloride in presence of potassium acetate form a color complex. The intensity of the color of the resultant mixture is directly proportional to the concentration of flavonoid present in the test solution.

Preparation of extracts of triphala guggulu

Triphala guggulu(1gm) was extracted with 100 ml methanol and filtered. It was collected in 100 ml volumetric flask and volume was made up with methanol. The same procedure was performed for each test samples of triphala guggulu.

Reagents

10% aluminum chloride
1M potassium acetate

Standards solution of Quercetin

Accurately weighed quercetin (100mg) was dissolved in distilled water and made up to 100 ml with distilled water in volumetric flasks. Aliquots of this solution were taken to prepared various working standard solution with concentration from 100µg/ml to 500µg/ml.

Calibration curve

A series of calibrated 10ml volumetric flasks were taken and appropriate aliquots of the different concentration of working standard solution (0.5ml) of quercetin were pipette out to each flask, 10% aluminum chloride solution(0.1ml), 1M potassium acetate solution(0.1ml), add distilled water(2.8ml) remained at room temperature for 30 min. the absorbance was measured at absorption maxima 415 nm, against the reagent blank prepared in the similar manner without the quercetin.

The liner correlation between these concentration (x-axis) and absorbance(y-axis) were graphically presented and the slop(b), intercept(a) and correlation coefficient(r) were calculated out for linear equation(y=bx+a) by regression using the method for least square.

Estimation of Total Flavonoid content in test solutions

The appropriate aliquots from extracts of each batch of triphla guggulu were withdrawn in 10 ml volumetric flasks separately. The color complex was developed in the similar manner as in calibration curve studies, replacing the quercetin with triphala guggulu extracts and the absorbance for aliquots of each sample was noted at 415 nm. The responding concentrations of triphala guggulu against respective absorbance were determined as quercetin using the calibration curve.

In vitro Antioxidant activity of methanolic extracts of lab and three marketed triphala guggulu formulation:[7,8]

Preparation of extract

For methanolic extract

1gm of each sample of triphala guggulu was extracted with 100ml methanol by Reflux for 1hr.The extracts was filtered and concentrated to dryness

Ferric Reducing Power Ability (FRPA) Assay[7]

Introduction:

Ferric reducing ability assay is a technique to determine the total antioxidant power interpreted as the reducing capability. The reducing power of plant extract was determined by the method of Oyaizu et al. The capacity of extract to reduce the ferric cyanide complex to the ferrous- ferric cyanide complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation.

The higher the absorbance of the reaction mixture the greater is the reducing power.

Chemicals& Reagents:

Phosphate buffer:1.7 gm of disodium hydrogen phosphate(Na2HPO4) and 0.6935 gm of potassium dihydrogen phosphate(KH2PO4) was dissolved in 50 ml of distilled water.

Potassium ferric cyanide(1%); 1gm of potassium ferric cyanide was dissolved in 100 ml of distilled water.

Tri chloro acetic acid(TCA)(10%): 10 gm of tri chloro acetic acid was dissolved in 100 ml of distilled water. Keep this in cool temperature

Ferric Chloride(Fecl3)(0.1%): 0.1 gm of ferric chloride was dissolved in 100 ml of distilled water.

Preparation of sample extracts:

The methanolic extract above mentioned was taken in range of 20-100 µg/ml in water.

Preparation of standard solution:

Ascorbic acid was as standard. Aliquots of 20-100 µg/ml in methanol were prepared.

Procedure:

Different concentration of plant extract in 1ml of distilled water was mixed with phosphate buffer(2.5ml) and potassium ferric cyanide(2.5ml) and the mixture was incubated at 50Cº for 20 min . 2.5 ml of 10% TCA was added to the reaction mixture which was centrifuged at 1000 RPM for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water(2.5ml) and FeCl3 (0.5ml) and the absorbance was measured at 700nm.

In vitro Antimicrobial activity of laboratory& three marketed triphala guggulu formulation:[6]

Materials and Methods:

Test organism and Inoculums:

Gram positive:- styphylococcus aureus.

Standard:- Kenamycin use as standard

Media:- Dehydrated nutrient ager media was used and was prepared in distilled water. The composition of the media was as given under-

Composition Of Nutrient Agar Medium

(1)Agar	15.0%
(2) Peptic digest of animal tissue	5.0%
(3) Sodium chloride	5.0%
(4) Beef extract	1.5%
(5) Yeast extract	1.5%
(6) Ph	7.4±0.2 at 25ºC
(7)Distilled water	1000ML

The medium was autoclaved at 15 Ibs per square inch pressure at 121ºC.

Preparation Of Media:- Dehydrated nutrient agar media(28gm) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask.It was heated on awater bath to dissolve the medium completely.

Sterillization Of Media:- The conical flask containing the nutrient agar medium was plugged with the help of non-absorbent cotton bung. The mouth of the conical flask and the cotton bung were properly covered with aluminium foil. The medium was then sterilization by autoclaving at Ibs per square inch pressure for 20 minutes.

Method:-

Cup and Plate Method.

The sterile nutrient agar medium at a temperature between 40º to 50º was immediately poured into the sterile Petri plates to give a depth of 3 to 4 mm ,by placing the plates on a level surface. The plates were then allowed to solidify. Each plate was then inoculated with 0.1 ml of the solution of test organisms prepared in water for injection. The wells in each plate were bored in the centre that was filled with 1000mg of triphala guggulu. The plates were then incubated at 37º for 24h. After incubation, zonal inhibition(inhibition around each well) was measured and this value was taken as an indicator for the antimicrobial activity.

RESULTS AND DISCUSSION

Estimation of total phenolic content by UV-spectroscopic method

The amounts of total phenolic contents in test sample was calculated using following regression equations:

$Y=0.0009x+0.0288(R^2= 0.998)$

Where,X=concentration of the sample

Y= absorbance

Estimation of total flavonoid content by UV-spectroscopic method

The amounts of total flavonoid content in test sample was calculated using following regression equations:

$Y= 0.0024x+0.0428(R^2= 0.995)$

Where, X= concentration of the sample

Y= absorbance

Ferric Reducing power Ability(FRPA) assay:

The reducing power of plant extract was determined by the capacity of extract to reduce the ferric- ferric cyanide complex to the ferrous – ferric cyanide complex. FRPA assay is one of the tests used to prove the ability of the formulation of triphala guggulu extract to reduce the ferric-ferric cyanide complex to the ferrous-ferric cynide complex. The obtained results are shown in fig 3 & 4. The methanolic extract of dabur triphala guggulu showed a significant reducing effect.

Antibacterial Activity Of Four Formulation Of Triphala Guggulu:

The results of antibacterial activity indicated the triphala guggulu formulation exhibited reasonable inhibitory activity aginst microorganisms under test.

Table:- 1 Data of spectrophotometric analysis for total phenolic content

Parameters	For total phenolic content
Wavelength for measurement	765 nm
Concentration range(mcg/ml)	100-1000
Regression equation	y=0.0009x+0.0288
Correlation coefficient	R^2=0.9971
Intercept	0.0288

Table :2 Data of UV-spectroscopy method for total phenolic content

Test solution	Total phenolic content as mg GAE/g
CU1	356mg
CU2	401 mg
CU3	356mg
CU4	384 mg

Table:-3 Data of spectrophotometric analysis for total flavonoid conent	
Parameters	**For total Flavonoid content**
Wavelength for measurement	**415 nm**
Concentration range(mcg/ml)	**100-500**
Regression equation	**y=0.0024x+0.0428**
Correlation coefficient	**R^2=0.995**
Intercept	**0.0428**

Table 4: Data of UV-spectroscopy method for total Flavonoid content	
Test solution	**Total Flavonoid content as mg QUE/g**
CU1	**2.7**
CU2	**3.2**
CU3	**3.45**
CU4	**2.6**

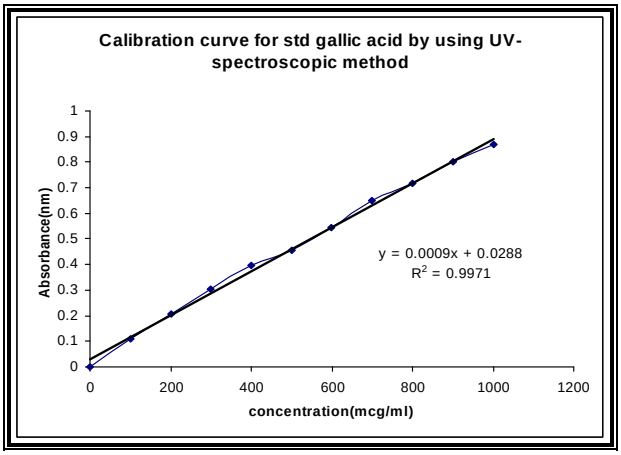


Figure:- 1 linearity curve for std.gallic acid by using UV- spectroscopy method

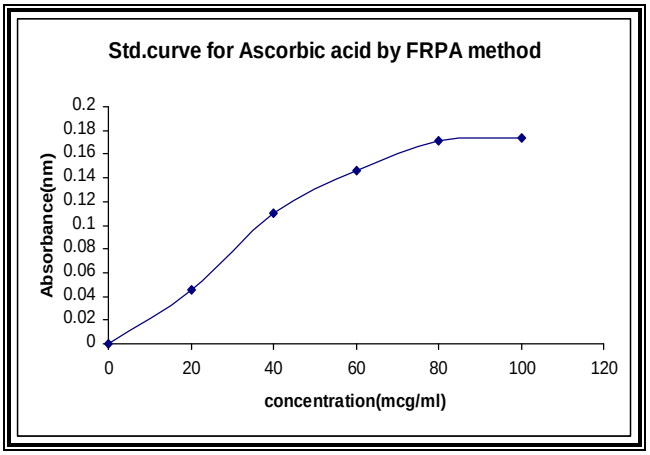


Figure:-3 % reducing effect of Ascorbic acid as a standard by using FRPA Method

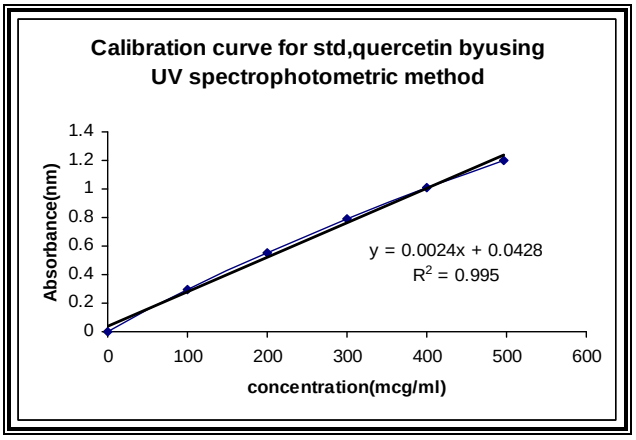


Figure:- 2 linearity Curve for std quercetin by using UV-spectroscopic Method

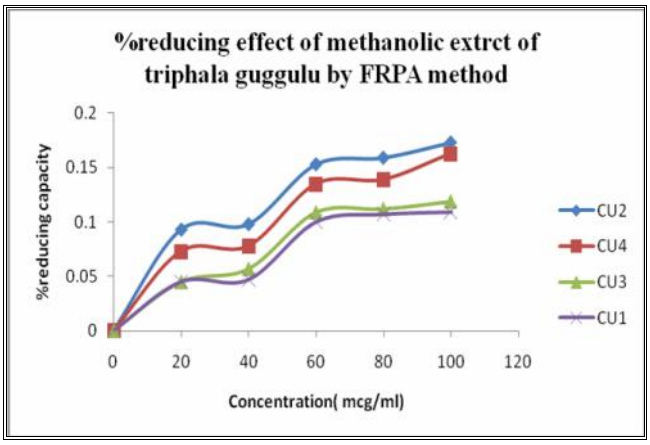
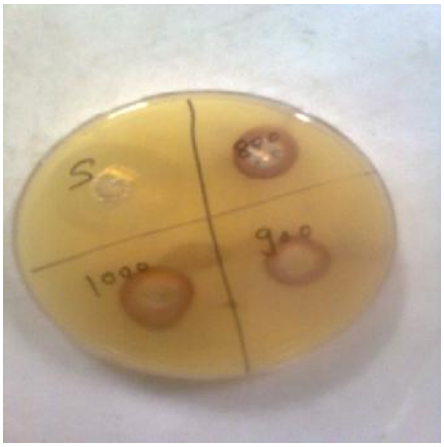
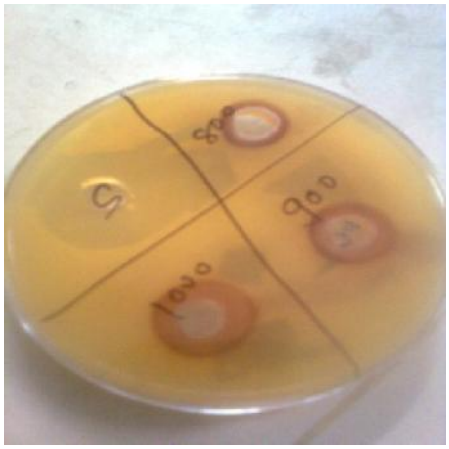


Figure:-4 % reducing effect of Methanolic extract of Four formulation of Triphala guggulu



CU1

CU2



CU3

CU4

Figure:-5 Antimicrobial activity of triphala guggulu with standard

CONCLUSION:

From results it is evident that triphala guggul of CU2sample of triphala guggulu has higher total phenolic constitutents compared to other three formulations followed by CU4 , CU3 and CU1.
From results it is evident that triphala gugul of CU3sample of triphala guggulu has higher Flavonoid content compared to other three formulation. And followed by CU2,CU1 and CU4
From results it is evident among four formulation CU2 sample of triphala guggulu have high reducing capacity followed by CU4,CU3,CU1.
From Result it is evident that CU2 sample of triphala guggulu has higher antibacterial activities compared to other three triphala guggulu formulation.

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