PROJECT WORK REPORT

ASHWAGANDHA AND TRIPHALA IMPROVE EFFECT OF EYESIGHT AND ANTICATERACT

Submitted To Gujarat Technological University

IN

B. PHARMACY

SEMESTER-VIII

BY

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Declaration

We here by declare that thesis entitles “Ashwagandha and triphala improve effect of eyesight and anticateract” it is a bonafide and genuine carried out by our. The results or observational data presented in this report are original.

26/04/2021
Place: Rajkot

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collage

ACKNOWLEDGEMENT

"Knowledge is in the end based on acknowledgement."

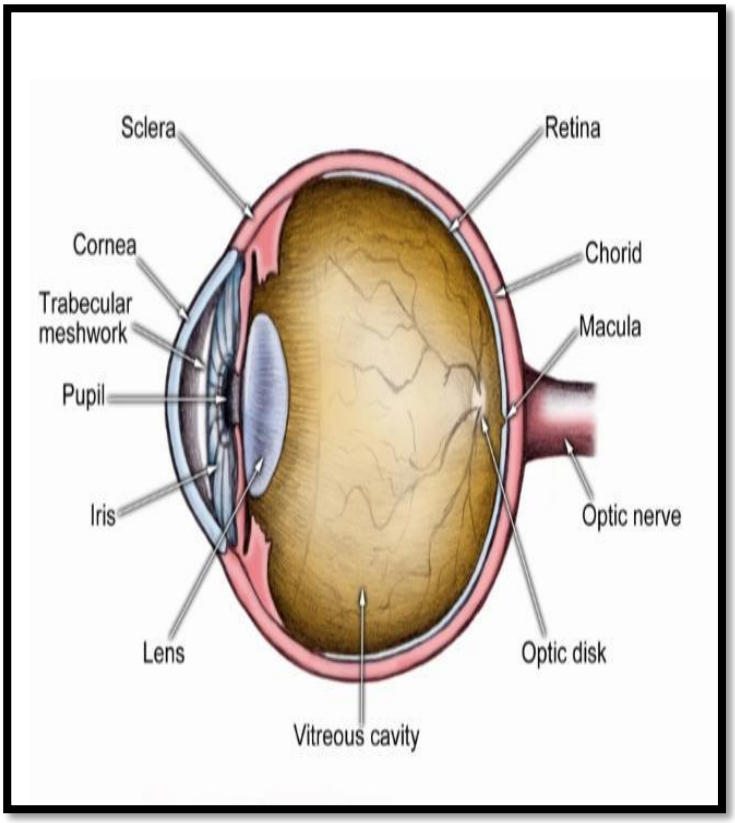
We warp up this project; We take this opportunity to express my thanks to all who helped to complete the task.

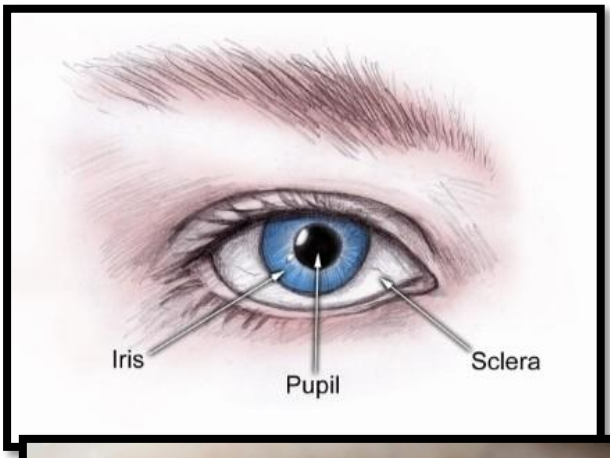
We take this opportunity to express my deep sense of gratitude to my guide MISS.REENA KORAT,M.PHARM., assistant professor of shree h.n.shukla institute of pharmaceutical education and research, for her guidance, valuable suggestions and liberal encouragement to complete this work successfully entitled "**ASHWAGANDHA AND TRIPHALA IMPROVE EFFECT OF EYESIGHT AND ANTICATERACT**"

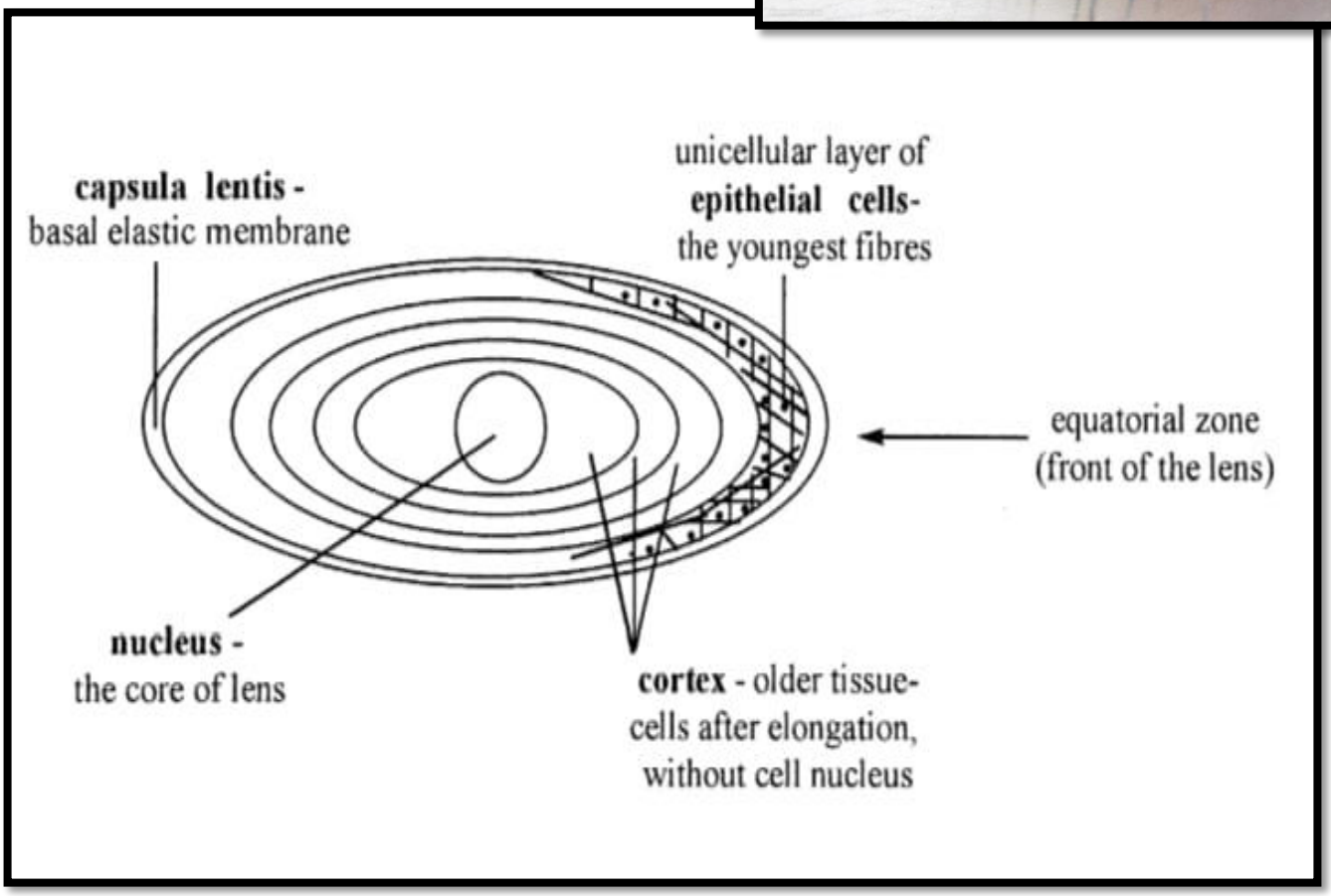
It's our privilege and honor to thank DR.DHARA CHAVDA,M.PHARM,Ph.D., principal, shree h.n.shukla institute of pharmaceutical education and research, for providing all the necessary facilities to do this project work.

INDEX:

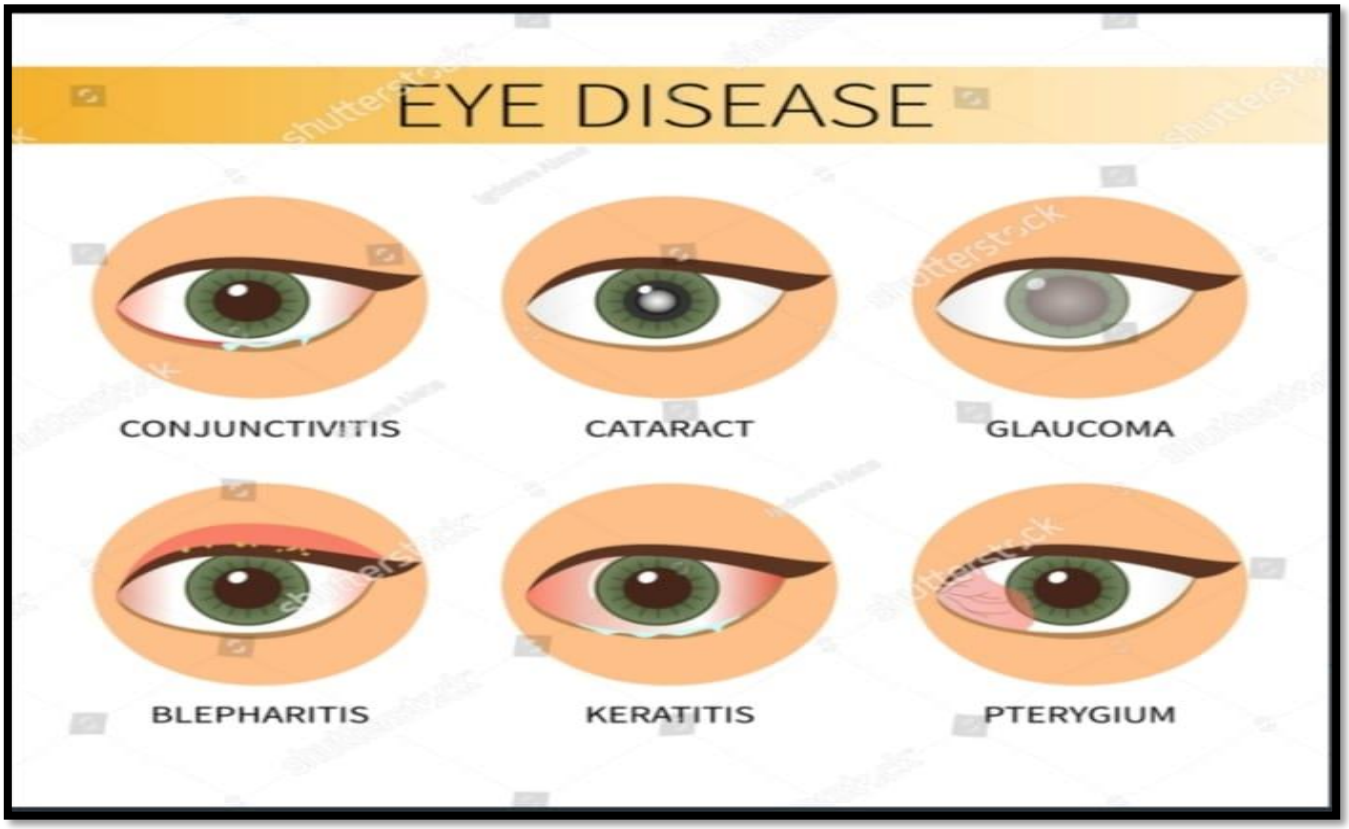
❖ <u>Structure of eye & Lence:</u>- INTRODUCTION

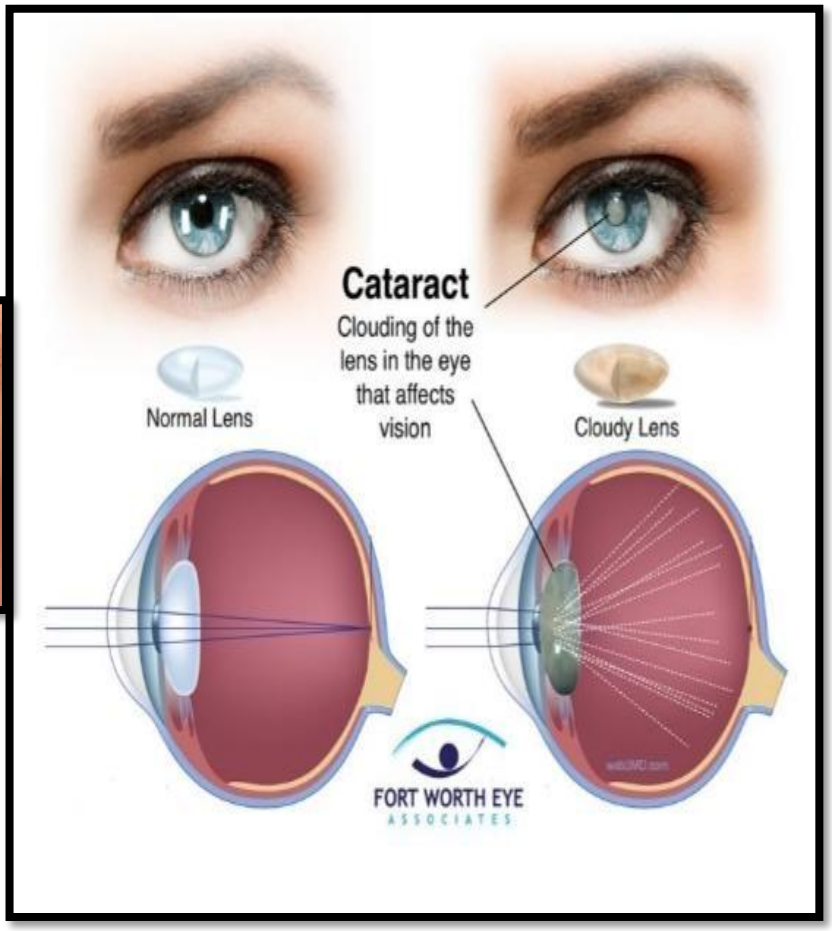






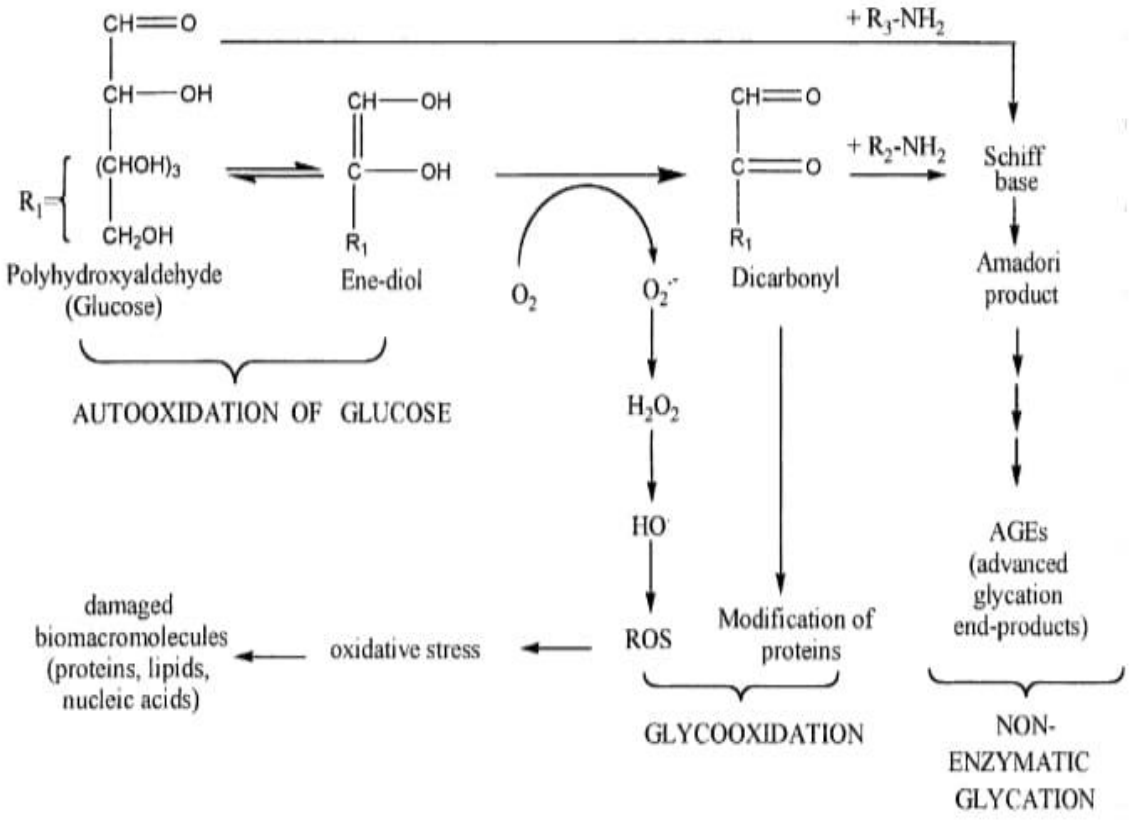
❖ Types of eye disease:- INTRODUCTION





❖ **Causes occur week eyesight and cataract:-**

- **Oxidative stress and diabetes mellitus**:-

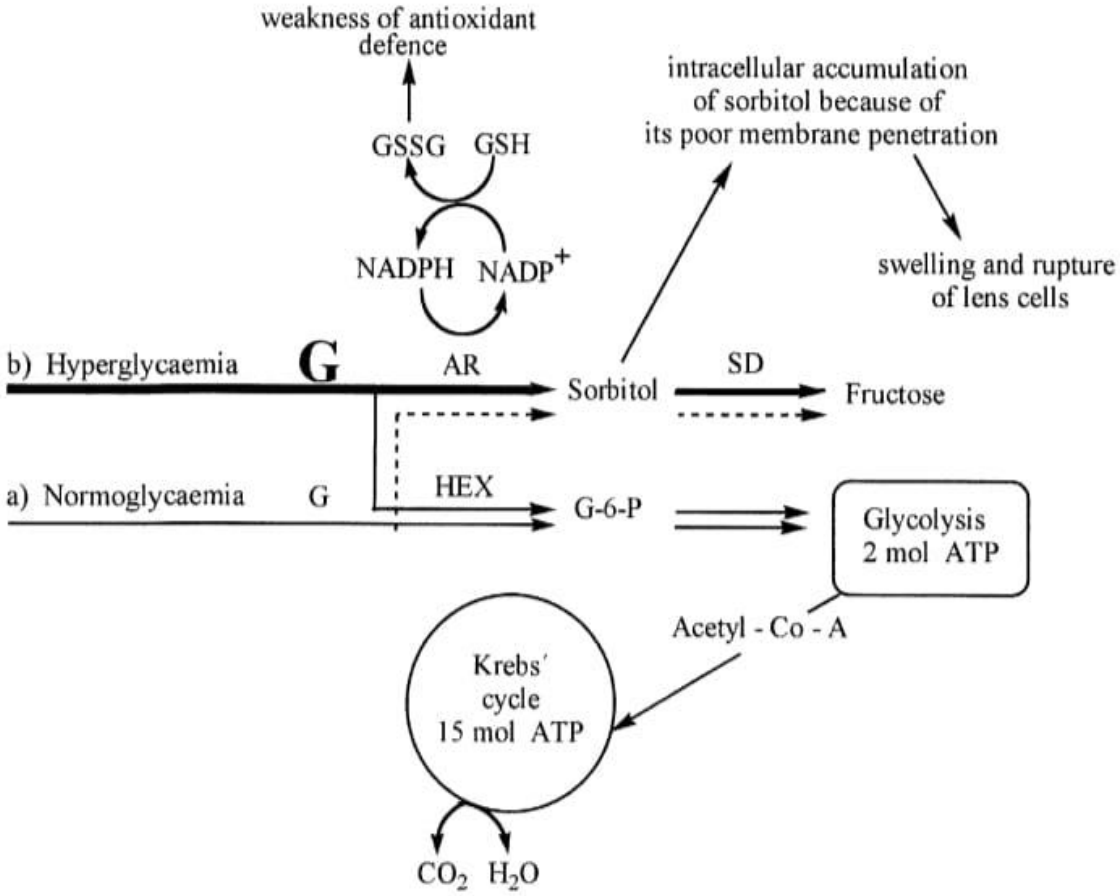


Where,

- o2=oxygen
- H2o2=Hydrogen peroxide
- Ho=Hydroxyl Radical
- Ros=Reactive oxygen species
- R3-NH2=Polypeptide chain

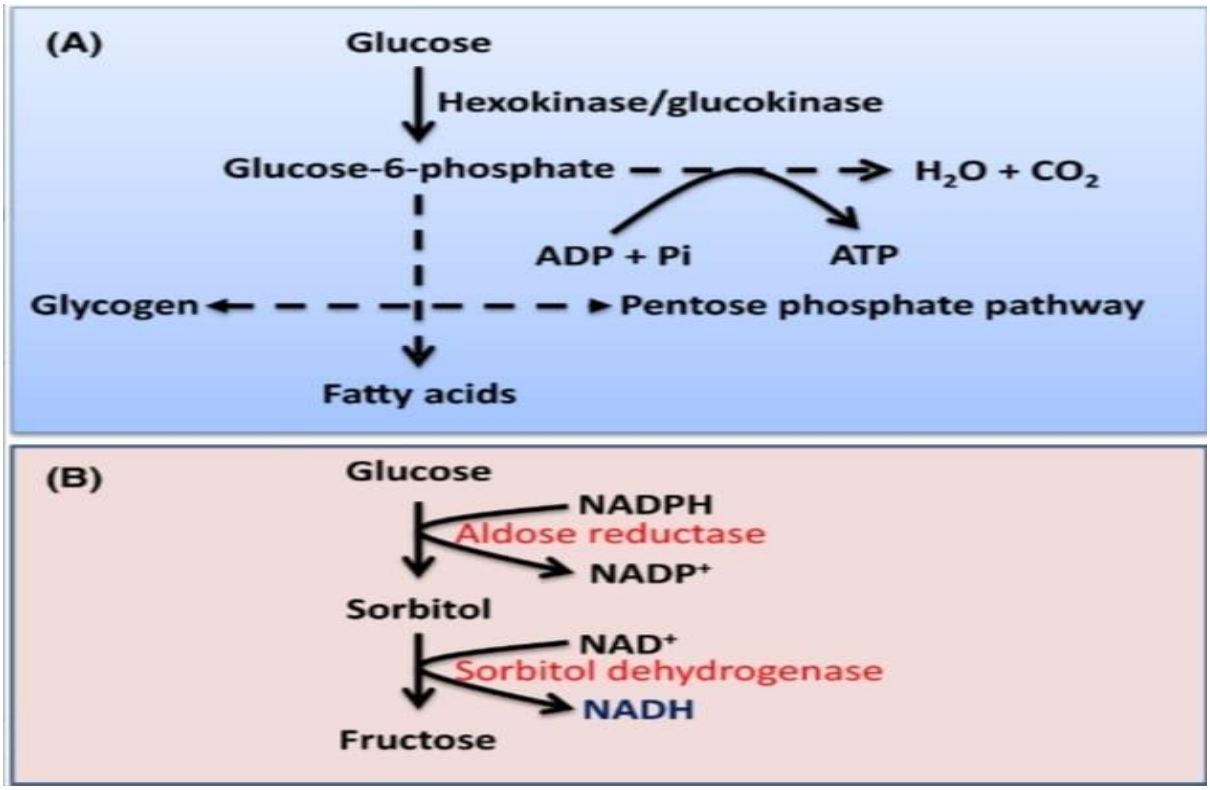
- Diabetes mellitus was found to be inextricably connected with increase oxidative stress both in diabetic human and hyperglycemia animal (Haynes) 1991; Cameron, Cotter.

- **<u>Polyval pathway week antioxidant properties:-</u>**



Where,

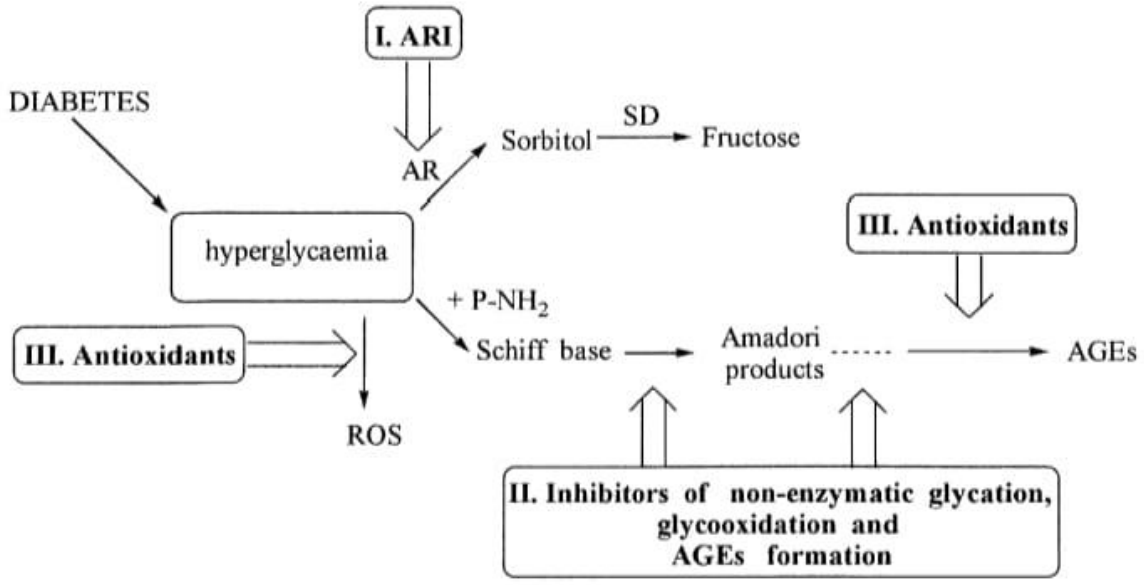
- G=Glucose
- G-6-p= Glucose-6-Phosphate
- AR=Aldose Reductase
- SD=Sorbitol Dehydrogenase
- HEX=Hexokinase
- ATP=Adenosine Triphosphate
- Acetyl==Co-A-Acetyl coenzyme A
- GSH=Reduced Glutathion



- NADH=Reduced Nicotinamide Adenine Dinucleotide
- NADPH-Reduced nicotinamide Adenine Dinucleotide Phosphate

- Under physiological condition, the bulk of glucose is metabolism through the glycolytic pathway and the pen-tose shunt. When hyperglycemia occurs, glucose disposal through these pathway tends to increase (pugliese, Fulton & Williamson, 1991). In addition , an increase amount of glucose is converted I to sorbitol by the enzyme AR via the poly pathway, normally operating for converting aldehydes into alcohol at physiological glucose concern-tractions (Williamson et al.1993). the glucose conversation into sorbitol by utilitizing NADPH result in the reduction of the NADPH/NADP+ ratio; sorbitol does not easily cross cell mem.

❖ **<u>Possibility of pharmacology of prevention of caterect:-</u>**



Where,

- ❖ AR=Aldose reductase
- ❖ SD=Sorbitol dehydrogenase
- ❖ ARIs=Aldose reductase inhibitor
- ❖ ROS=Reactive oxygen species
- ❖ AGEs=Advanced glycation end prods
- ❖ P-NH2=Protein site of drug action

❖ **Herbal component:-**

Triphala:

1. Amalaki (amala)
2. Hauitaki (harade)
3. Bibhitaki (beheda)

1. Amalaki:-

- Synonums – emnlica , amala
- Biological Source– this consists of dried as well as fresh fruit of plant emnlic officinalis
- Family _ euphoubiaceae
- Geographical Source – small-medium sized trace found in all india , shrilanka and Myanmar
- Chemical Constitute– vitamin- c , Crucial acid , alagic acid

Morphological characteristics :

- Colour : green changing to light yellow or brick red when matured
- Odour : none
- Taste : sore and astaingent
- Shape : globular shape
- Size : 1.5 to 2.5 cm in diameter

Used

- Diuretic
- Diabetes
- Laxative
- Improve eye sight – antioxidative
- Dried fruits used in haemouahage

2.Haritaki:-

- Synonyms : chebulic nyuobalan , haude, haritaki

- Biological Source – myrobalan contain dried , ripe and fully matured fruits of Terminalia chebula
- Family : combuetaceace
- Geographical source : mainly South asia fruits india and Nepal , asssam ,MP, bihar Maharashtra, west Bengal
- Chemical Constitute: ellagic, gallic acid content

Used :

- Laxative
- Astaigent
- Stomach .

3.<u>baheda</u> :-

- Synonyms: bayada , bibhitaki , bekeuic , myrubalan

- Biological Source: it consists of dried ripe fruits of the plant Terminalia beleaica
- Family: bibitaki fruits contain several phytochemical constituent
    - Beta – sitosterol
    - Gallic acid
    - Ellagic acid
    - Ethyl jallate
    - Cardiac glycoside

Morphological characteristics :

- ❖ Colour : fruits are dark brown to black
- ❖ Odour : none
- ❖ Taste : Astringent
- ❖ size : 1.3 to 2 cm length
- ❖ Shape : fruits are globular and obscurely
- ❖ Uses : astringent , diaruboea , constipation , demulcent and paugative

❖ **<u>Action of Triphala:-</u>**

Three content

Amalaki (amala)

Hauitaki (harade)

Bibhitaki (behead)

- Mixed these three component
- High antioxidative & antidiabetic activity for eye health
- Staees reducing potential of taiphala
- Radiopastective effect of triphala
- That next anti-tumjual activity
- Also used constipations activity and detaxifaction

- It’s action effective beneficial activity of taiphala

❖ **Our new component :-**

- **Ashwagandha :**

- Our new herbal component
- Biological source : dried root & stem based on withania somnifera
- Family : solanaceae
- Geographical source : congo , Jordan, Pakistan, Egypt, gujrat , Punjab, rajastan , UP, MP
- Harvesting in last march

- Chemical constitute:
  steroidal alkaloids : anfeain , withanine, tropine , choline , anahygaine
  Steroidal lactone : withanolides , withafeuine , withefeuin-A , withanine

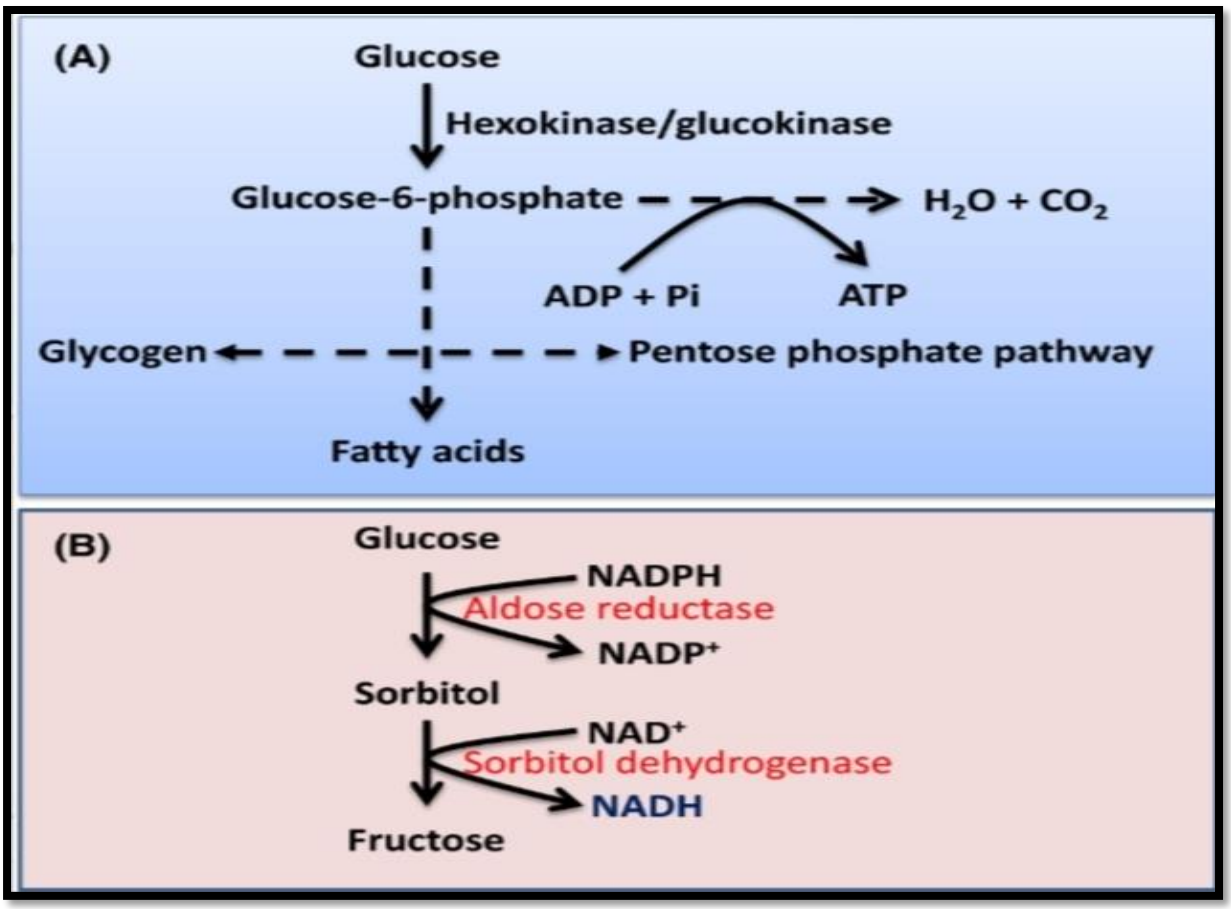
Morphological characteristics :

- Size : length 10-17 mm
  Width 6-12 mm
- Shape : conical or cylindrical , straight , unbranched
- Colour : acrayish yellow
- Odour : fainty pungent and characteristic
- Taste : mucilaginous , acrid and bittery

Uses :

- High antioxidative effect
- Decrease glucose level
- Anti – stress activity
- Anti tumour activity
- Widely used sex stimulated
- Sedative and hypnotic
- Hypotensive , respiratory stimulant action with buadyudia

Mechanism of action Ashwagandha :-



- Ashwagandha in poyal pathway antioxidant & antidiabetic effect
- Ashwagandha prove also aldose reductase inhibitors
- SDP glucose convert to sorbitol to fructose
- Say high antictaterect effect prove Ashwagandha
- So its proven activity weak eye sight & conductivities related problems

❖ Compatibility of Ashwagandha and triphala together

- Ashwagandha is a single herb whereas taiphala is a combination of 3 herbs
    - Awala
    - Hauitaki
    - Bibhitaki
- All these heabs are compatible with Ashwagandha
- All the herbs of triphal (awala, hauitaki and bibhitaki) are astaingent and warm in nature .
- Ashwagandha is also straigent
- All the above herbs are rasayana or adaptogens
- They help to normalize all the function of the baby
- Also these are same activity antioxidant and antidiabetic effect
- And these also compatible formulation of powder and liquid dosage forms
- Beside these are no drug interaction or herb incompatibilities reported among any these herbs
- A combination of Ashwagandha with triphala is the right combination , especially for a person suffering from debility and chronic constipation.
- Ashwagandha & triphala both are same as activities And proven effective good foe eye sight problem like
    - Glocoma
    - Cataract

- Day eye
- Keratoconjuctive
- Trachoma
- Pain eyes to relives

❖ **Quality control parameters herbal formulation:-**

I. **Physical parameters**: it include colour, appearance, odour, clarity, viscosity, moisture, content, ash values, pH, disintegration time, friability, hardness, flow property, flocculation, sedimentation and settling rate.
II. **Chemical parameters:** it include colour, limit tests for heavy metal, extractive values, chemical assays for active constituents, etc.
III. **Chromatographic of herbals:** chromatographic analysis can be carried out using TLC, HPLC, HPTLC, GC, UV, Fluorimetry and GCMS etc.
IV. **Microbiological parameters :** it includes total viable content, total mold count, total entero-bacteria-ceae and their count. Morphology. The in this present study one of the important ayurvedic triphala churna was standardized. Churna are preparations comprising of fine powders of drug and may be simple or compound. Simple churna is due to the fact that therapeutic value of most of the substance greatly increases when they are reduced to very fine state of subdivision

Triphala & Ashwagandha churna and its composition

Emblica officinalis is effective in the treatment of hepatoxicity, amlapitta and in dyspepsia. the fruits exhibit hypolipiadaemic and anti atherosclerotic effects in rabbits and rats. the fruit extract has anti-mutagenic activity on certain directly acting mutagens in some strains of salmoellatyphimurium. The extract of alma also has antimicrobial

properties. Amalaki & Ashwagandha is an antioxidant with free radical scavenging properties. Which may be due to the presence of high levels of super oxide dismutase. lignin isolated from Terminalia bellirica were shown to possess anti-hiv antimalarial, protective effect on liver and anti-fungal activities. the fruit pericarp of Terminalia chebula showed cytoprotective activity cardio tonic activity, anti-mutagenic activity and antifungal properties.

❖ Materials and methods

1. Collection of herbal material

The ingredients used in the Ashwagandha and triphala churna are amlaki (embellica officinalis),bibhitaka (Terminalia chebula) were purchased from local market. Drugs are cleaned and dried properly.

Drug are kept separately and powdered. They are sieved using 80-mesh sieve and each one of then powdered and weighed separately and then mixed kept in air tight containers in cool and dry place along with dabur triphal churna and Ashwagandha churna also purchased for the reference.

2. Chemical and requirements

Rotary vacuum evaporator (equitron roteva) ,water bath (kokate; sofawora 1993) automatic centrifuge (medica instrument MFG Co .) digital cat cam camera, semi-automated biochemistry analyser , UV spectrophotometer (1800 shimadzu corporation , Kyoto, japan). Chemical like methanol ,lead nitrate ammonium ferrous sulphate ,hydroxyl amine hydrochloride , silica gel G were obtained from S.D. fine chem ltd. Mumbai , india.

3. Preparation of aqueous and ethanolic extracts of triphala and Ashwagandha mixed churna

A weighed quantity of powder (500gm .) was passed into sieve no. 40 and subjected to aqueous and ethanolic with distilled water and 90% ethanol and kept at room temperature for 7 days with occasional stirring the extract was filtered . the aqueous and ethanolic extracts were concentrated in water bath extraction was dried under reduced pressure using rotary vacuum evaporator . then the both dried extracts are preserved in the desiccator and used for further purpose.

4. Pharmacognostical evaluation of powdered crude drug

Microscopic evaluation

Macroscopic study was carried out by colour, odour and teste for samples in of the churna .

Determination of powder flow property

Physical properties of lab and market formulation were determined by the parameters described below :

[A] bulk and tap density

Both bulk density (BD)and tapped density (TD) was determined as per USP. A quantity of 10 gm powder blend was introduced in to 25 ml measuring cylinder. After that initial volume was noted and the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no further change in volume was noted.BD and TD were calculated using the following equations.

BD=Weight of the powder bled/untapped volume of the packing

TD=weight of the powder blend/tapped volume of the packing

[B] carr`s index (compressibility index)

The compressibility index of the powder blend was determined by carr`s compressibility index. The formula for carr`s index is as below

Carr`s index (%) = [(TD-BD x 100]/BD

[C] Houser's ratio

The formula for Houser's ratio is as below :

housner's ratio = tape density / bulk density .

[D] angle of repose

Angel of repose :- h/r

The angle of repose of powder blend was determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on the surface. The calculated using the following equation

Microscopical examination

Powder characteristics of the drug were studied under the microscope. The stained and unstained slide prepared were examined and unstained slide was prepared and the characters were examined and photographed using digital CCD camera.

Procedure : powder was boiled with clarifying reagent chloral hydrate for few minute . after boiling .the powder was mounted on the slide with lactophenol 50% glycerine for the unstained slide preparation , powder was stained with the phloroglucional and conc HCL and mounts with 50% glycerine and covered with the cover slip. The stained slide was also prepared using iodine solution for starch grains .the slide were examined under the microscope.

Physico-chemical parameters ash value

1) Total ash value

3 gm of the powdered drug was accurately weighed and taken in a silica crucible which was previously ignited and weighed. The powdered drug was spread as a fine even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing the temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed, repeated for constant value. The percentage of the total ash was calculated with reference to the air dried drug.

2) Acid insoluble ash

The ash obtained as described in total ash was boiled with 25 ml of 2 N Hcl for 5 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash transferred into a silica crucible was ignited and weighed. Repeat experiment to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

3) Determination of Sulphated Ash value

About 3 gm. of accurately weighed air dried powdered drug taken in silica crucible which has previously ignited and weighed. Then ignited gently at first until the drug was thoroughly charred. The crucible was cooled , residue was moistened with 1 ml of concentrated Sulphuric acid heated gently until the white fumes were no longer evolved and ignited at 800°C ± 25°C until all the black particles has disappeared. The crucible was allowed to cool. Few drops of Sulphuric acid were added again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight (difference is not more than 0.5 gm. between two consecutive readings). The percentage of

Sulphated ash was calculated with reference to the air dried drug. All the ash values were calculated and recorded

Loss on drying

2 gm of powdered drug was taken in tarred china dish. Dried in the oven at 100°C or 105°C, cooled in a desiccator and watch. After that the loss was recorded as moisture. The procedure was continued for at least two common readings.

Extractive values

1) Determination of alcohol-soluble extractive

Macerated 5 g of shade dried coarse powder of leaves with 100 ml of alcohol (90%) in two separate closed flasks for 24 hrs, shaking frequently during first 6h and allowed to stand for another 18 hrs. Filtered rapidly, taking precautions against loss of alcohol. Evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. Calculated the percentage of alcohol soluble extract with reference to the shade dried drug.

2) Determination of water soluble extractive

The same procedure was followed as directed for the determination of the alcohol soluble extractive by using chloroform water instead of alcohol. The extractive values of drugs were calculated and recorded.

Limit test for Heavy metal of Triphala & Ashwagandha churna

Preparation of Lead nitrate stock solution – Accurately weighed 0.1598 g of lead nitrate was diluted in 100 ml of water to which had been added 1 ml of nitric acid, then diluted with water to 1000 ml.

Standard lead solution–10.0 ml of lead nitrate stock solution was diluted with water to100ml. Each ml of standard lead solution contains the equivalent of 10 μg of lead.

Preparation of Standard solution- 2 ml of standard lead solution was pipetted out into a 50 ml Nessler cylinder and diluted with water to 25ml. then pH Adjusted with dilute acetic acid or dilute ammonia solution between 3.0 and 4.0, then diluted with water to about 35 ml and mixed.

Preparation of Test solution- Accurately weighted 2 gm of Churna (lab and market Churna) was added in to suitable crucible. Sufficient Sulphuric acid was added to wet the sample and ignited carefully at a low temperature until thoroughly charred. 2 ml of nitric acid was added to charred the mass and then five drops sulphuric acid was added. It was heated cautiously until white fumes are no longer evolved Ignited, in a muffle furnace, at 500ºC to 600ºC until the carbon is completely burnt off. It was cooled and 4 ml of hydrochloric acid was added, covered and digested on a water bath for 15 min.The residue was moisten with one drop of hydrochloric acid. 10 ml of hot water was added and digested for two minutes. It was cooled and ammonia solution was added, drop wise, until the solution is just alkaline to litmus paper, it was diluted with water to 25 ml and pH was adjusted with dilute acetic acid between 3.0 and 4.0. The Solution was filtered. The crucible was rinsed with 10 ml of water, Filtrate were combined in a 50 ml Nessler cylinder, diluted with water, to about 35 ml and mixed.

Procedure: To each of the cylinders containing the standard solution and test solution respectively 10 ml of freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50 ml, allowed to stand for five min. It was observed downwards over a white surface, the color produced in the test solution and standard solution were observed and compared.

Preliminary Phytochemical Studies

Introduction: Plants may be considered as biosynthetic food laboratories in which various compounds are synthesized such as carbohydrates, proteins, lipids, flavonoids, alkaloids, volatile oils, tannins etc. that exerts physiological effects. The medicinal value of any drug depends on the nature of chemical constituents present in it, which is referred as active constituent. In order to detection of active

constituents plants are needed to be subject to phytochemical screening according to the standard procedures.

Qualitative Phytochemical Analysis

Test for alkaloids

A small portion of the solvent free extract was stirred separately with a few drops of dilute HCL and filtered. The filtrate was tested with various reagents

Dragendrof's Test: To the 1 ml of extract add 1 ml of reagent (potassium bismuth iodide). An orange red precipitate indicates the presence of alkaloids.

Mayer's Test: To the 1 ml of extract add 1 ml of reagent (potassium mercuric iodide). Whitish yellow or cream color precipitate indicates the presence of alkaloids.

Hager's Test: To the 1 ml of extract add 1 ml of reagent (saturated aqueous solution of picric acid). Yellow color precipitate indicates the presence of alkaloids.

Wagner's Test: To the 1 ml of extract add 1 ml of reagent (iodine in potassium iodide). Reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids

i) Shinoda's test: the alcoholic extract was treated with magnesium foil and conc. HCL gives intense cherry red color, indicates the presence of flavones.

ii) Alkaline Ammonium Test: The Ethanolic extract is treated with 10% sodium hydroxide solution and ammonium was added. Dark yellow color indicates the presence of flavonoids.

Test for proteins and amino acids

i) Biurets test: Add 1 ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced and then add

to the 1ml of the extract. Formation of pink or purple violet color indicates the presence of proteins.

ii) Ninhydrin test: Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1%solution in n-Butanol) to the small quantity of extract and heat. Development of blue color reveals the presence of proteins and amino acids.

iii) Xanthoproteic test: To 1 ml extract, add 1ml of concentrated nitric acid, a white precipitate is formed, it is boiled and cooled. Then 20% sodium hydroxide or ammonia is added. Orange color indicates the absence of aromatic amino acids.

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for steroids

Two ml of acetic anhydride was added to 0.5 g Ethanolic extract of each sample with 2 ml H2S04. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of extract was mixed in 2 ml of chloroform and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

Test for Fats and Fixed Oils

Press a small quantity of extract and powder separately between two filter papers. Oil stain's on the paper indicates the presence of fixed oil.

Test for Volatile oils

The extract gives pink cooler with Sudan red III solution

Quantitave phytochemical estimation of Triphala Churna

Estimation of Total Tannins

Total tannin content in water extract of Amla, Baheda, Harde and Ashwagandha Churna were measured by redox titration method as per reported method.

Reagents: 0.1 N KMnO4: It was prepared by 3.16 gm KMnO4 was dissolved in 1000 ml distilled water. 0.1 N Oxalic acid: it was prepared by 0.63 gm oxalic acid dissolved in 100 ml distilled water.

Preparation of Churna and its ingredients extract: 1 gm powder of Amla, Baheda, Harde and Ashwagandha Churna were extracted with 100 ml distilled water by heating at 70-80˚C for 1 hr separatly. Extract was filtered and volume adjusted to 100 ml in volumetric flask.

Standardization of KMnO4 : 25 ml of 0.1 N Oxalic acids was transfer in 100 ml conical flask. 25 ml of water, 5 ml of concentrated H2SO4 was added in to conical flask and heated at 70˚C. 0.1 N Oxalic acid was titrated with 0.1 N KMnO4. End point was characterized by change color of solution from colorless to pink.

Procedure: 10 ml of extract from stock solution was transferred into 500 ml conicalflask.10 ml indigo carmine was added in flask as indicator then volume was adjusted up to 300 ml. Solution was heated at 70˚C for 20min. Solution was titrated with 0.1 N KMnO4. End point was characterized by change color of solution from colorless to pink.

Factor: 1 ml of 0.1 N KMnO4 = 0.004157 gm. of total tannin calculated as tannic acid.

Estimation of Total Phenolic Content

The phenolic content in the water extract of Amla, Baheda, Harde and ashwagandha Churna was measured according to reported method.

Preparation standard stock solution: 100 µg/ml Gallic acid standard stock solution was prepared by 10mg Gallic acid dissolved in methanol and makeup volume up to 100 ml with methanol in volumetric flask.

Preparation of reagent:

[A] Folin ciocalteu reagent: Folin ciocalteu reagent: distilled water (1: 2) solution was prepared.

[B] 20% sodium carbonate solution: 20 gm of anhydrous sodium carbonate was dissolved in 100 ml of distilled water.

Preparation of Churna and its ingredients extract: 1gm. powder of Churna and ingredients were extracted with 100 ml distilled water by heating at 70-80˚C for 1h separately. Extract was filtered and volume adjusted to 100 ml in volumetric flask.

Procedure: 1ml of the samples solution was transferred into 25 ml volumetric flask separately. From the standard stock solution of 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0 ml were transferred into 25 ml volumetric flask which gives 2, 3, 4, 5, 6 and 8 µg/ml concentrationsrespectively.10 ml of water and 1.5 ml of Folin-ciocalteu reagent was added into each volumetric flask. The above mixture was kept for 5min and then 4 ml of 20% sodium carbonate solution was added. Volume was made up to 25 ml with distilled water. These mixtures were kept for 30 min and absorbance of blue color was measured at 765nm. Percentage of total phenolic was calculated from calibration curve of Gallic acid plotted by using the above procedure and total phenolic were expressed as % Gallic acid.

Estimation of Total Flavonoid content

Total flavonoid content in our Churna was measured by the aluminum chloride colorimetric assay.

Preparation of Drug Extract: 1g of Churna was weighed accurately and extracted with ethanol and volume is made up to 100 ml.

Preparation of Standard solution: Standard stock solution of Quercetin 1000 µg/ml was prepared by dissolving 10 mg in 10 ml. ethanol From this standard solution were prepared of concentration ranging 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml.

Procedure: An aliquot (1 ml) of extracts and standard solution was added to 10 ml volumetric flask containing 4 ml of distilled water. To

this 0.3 ml 5 % $NaNO_2$ were added. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content mg quercetin equivalents (CE)/100 G fresh weights. All samples were analyzed in triplicates.

Estimation of Total Alkaloid content

Preparation of std. calibration curve: Dissolved 100 mg Piperine in 10 ml chloroform and make up volume up to 100 ml with methanol. The spectrophotometric analysis carried out by followed 10 ml of each 5, 10, 15, 20, 25, 30 and 35 µg/ml concentration of Piperine was made proper dilution and taken into separating funnel in which added 5 ml acetate buffer and 3ml 0.05% methyl orange solution and shake well. The complex formed was extracted thrice (10+10+5 ml) with chloroform.

The complex formed was transferred to another separating funnel containing 25 ml of 1 M HCL. The dye liberated into hydrochloric acid from the complex was measured against a blank at 530nm using spectrophotometer (UV1800). Blank was prepared by same method without addition of standard.

Preparation of sample solution: Accurately Weighed 1 gm. Composition Churna powder lab and marketed Churna were taken, moistened with 10% ammonia (2ml), dried and refluxed and refluxed with chloroform (50ml) for1hr. This mixture was filtered, filtrate concentrated volume was adjusted to 25 ml with chloroform. Measured volume 0.5 ml of this extracted was taken and diluted to 10 ml with chloroform in volumetric flask and further treated with reagents as described above.

Estimation of Iron content (%)

The % Iron content in Churna powder lab and marketed Churna was measured by the colorimetric assay.

Preparation of Standard stock solution: Accurately weighed 25 mg Ammonium Ferrous Sulphate wad dissolved in 100 ml distilled water.

Preparation of Test solution of Churna (lab and market sample): Accurately weighed 100 mg of material was dissolved by heating with 100 ml dilute hydrochloric acid. The temperature was not allowed to exceed 50°C. After half an hour sample was filtered. 10 ml from this filtered solution was taken and diluted up to 250 ml with distilled water.

Procedure: An aliquot (2, 4, 6, 8 and 10 ml of standard solution and 25 ml of test solution) was taken in 50 ml volumetric flask each. To this 4 ml of 10 % Hydroxylamine hydrochloride solution and 4 ml of 0.3 % O-Phenanthroline solution were added in to each volumetric flask. It was swirled and allowed to stand for 10 min. The total volume was made up to 50 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 515nm.Percentage of Iron was calculated from calibration curve of Standard Iron solution plotted by using the above procedure

❖ **TLC fingerprinting of Churna :-**

TLC fingerprinting for Tannins

Stationary phase: Aluminum-backed silica gel 60 F254 plates (E. Merck) Solvent system: Toluene: Ethyl acetate: formic acid: methanol (3:3:0.8:0.2) Chamber saturation: 30 min.

Standard solution: Standard Gallic acid dissolved in methanol.

Test solution: Churna (laboratory and market samples) and its ingredients (Amla, Baheda, Harde & Ashwagandha) were extracted with methanol by cold maceration and methanol extracts were used for TLC fingerprinting.

Detection: detection carried with these wavelength's UV 254nm UV 366nm before and After Spraying with 5% FeCl3reagent.

TLC fingerprinting for alkaloids

Stationary phase: Aluminum-backed silica gel 60 F254 plates (E. Merck)

Solvent system: Benzene: Ethyl acetate (2:1)

Chamber saturation: 30 min

Standard solution: Standard Piperine dissolved in methanol.

Test solution: Churna (laboratory and market samples) and its ingredients were extracted with methanol by cold maceration and methanolic extracts were used for TLC fingerprinting.

Detection: detection carried with these wavelength's UV 254nm UV 366nm before and after Spraying with Dragendrof 's reagent.

In vitro Antioxidant activity of Churna25, 26

Method: 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity

Preparation of Test and STD sample: STD solution: Ascorbic acid was used as standard. Aliquots of 5- 30 mcg/ml in DMSO Test solution: Ethanolic extract of Amla, Baheda. Harde & Ashwagandha, were taken in range of 5- 30 mcg/ml in DMSO. Ethanolic extract of Churna (lab and market sample) were taken in range of 5- 50 mcg/ml in DMSO were prepared.

Procedure: 2ml of DPPH solution was added to 2ml DMSO and absorbance was taken after 30 minute at 517 nm for control reading. 2ml of DPPH were mixed with 0.6 ml of Different concentrations of sample and standard and add 2ml of DMSO. The mixture was kept in dark for 30 minutes and absorbance was measured at 517 nm after 30min.

The % reduction: % scavenging = (A control – A test / A control) × 100

Where, A test is the absorbance of the tested sample after 30minutes. A control is the absorbance of Control sample. IC50 is the concentration required to reduce % reduction by 50%.

Cell Culture: Human colorectal carcinoma (HCT116) cell line obtained from the American Tissue Culture Collection (ATCC) was used for the in-vitro assay and grown in Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 2gm of sodium bicarbonate. The pH 7.4 was maintained and the cells were incubated at 37°C with 5% CO2 in humidified incubator.

Thawing and Revival: Cryovials containing the frozen cells from liquid nitrogen storage were quickly thawed (< 1 min) by gently swirling the vial in the 37°C water bath. Thawed cells were transferred to a sterile tube containing required amount of medium corresponding to the cell lines and inverted for uniform distribution. The cell suspension was centrifuged at 1200g for 5 min. Clear supernatant was checked for visibility of the complete pellet, re suspended in complete growth medium and transferred to T-25 flask under the recommended culture environment (5% CO2 at 37°C). Growth was monitored and cells were trypsinized and sub cultured once they reached a confluence of 70-80%.

❖ In vitro Cytotoxic Activity by MTT assay:-

The cell lines were prepared and cryopreserved using reagents such as DMSO which preserve the cell during freezing. DMSO is toxic at room temperature. The freezed ampoule is brought to room temperature by slow agitation (thawing). The freezed cryo-vials plunged into the water bath and is rapidly thawed until it gets liquefied. Solution, centrifuged with saline for 10 mins to remove the DMSO. The saline is discarded and aliquot is taken for cell counting, cell viability and for sub-culturing. MTT assay is a quantitative colorimetric assay for measuring cellular growth, cell survival and

cell proliferation based on the ability of living cells. The assay was carried out using (3-(4, 5- dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT)). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. The result of In vitro cytotoxic activity of both extracts of Churna on Breast cancer cell lines.

Preparation of Herbal extract for the assay

0.5 ml of stock (100 mg/ ml) herbal extract was dissolved in 4.5 ml of DMSO for a concentration of 10 mg/ml. The fresh working suspension was filtered through 0.45µm membrane filter prior to the assay. Using the 1 mg/ ml concentration herbal extract, nine serial doubling dilutions of the extract of 500µl each was prepared in DMSO to get the concentration of the extract as indicated and the diluted extracts will be transferred to 10 wells of a 12 well culture plate. 500 µl of 48h culture of MCF 7 cell lines at a concentration of 105 cells/ ml was added to each well. Two control wells received only cell suspensions without plant extract. The plate was incubated in a humidified CO2 incubator at 37°C for 4-6h. The plate was microscopically examined for confluent monolayer of cells, turbidity and toxicity.

Assay Process

After incubation, the medium from the well was aspirated carefully and then discarded. Each well was washed with Eagle's Minimum Essential Medium (EMEM) without Fetal Calf Serum (FCS). 200 µl of MTT solution (5mg MTT/ ml of PBS, pH 7.2) will be added to each well. The plate was incubated for 6-7 h at 37°C in a CO2 incubator with 5% CO2. After incubation 1 ml of DMSO was added to each well and mixed with pipette and left for 45s at room temperature. Purple formazan was formed in the wells. Cell control and solvent controls

were included in each assay to compare the full cell viability in cytotoxicity and antitumor activity assessments. The suspension was transferred to a spectrophotometer cuvette and the optical density (OD) was measured at 540nm using DMSO as blank. The % cell viability was calculated with the following formula

Cell viability %: =Mean OD of wells receiving each plant extract dilution / Mean OD of control wells × 100.

Determination of IC50: IC50, the concentration of compound required to inhibit 50 % cell growth, was determined by plotting a graph of Log (concentration of Extract) vs % cell inhibition. A line drawn from the 50 % value on the Y axis meets the curve and interpolate to the X axis. The X axis value gives the Log (concentration of the compound). The antilog of that value gives the IC50 value. Percentage inhibition of novel compounds against all cell lines was calculated using the following formula:

$$\text{Cell survival} = \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

Where, At = Absorbance of Test, Ab= Absorbance of Blank (Media), Ac= Absorbance of control (cells) % cell inhibition = 100 − % cell survival

Statistical analysis: The results were expressed as mean ± SEM. The Statistical comparision made by Students t-test. Only those mean values showing statistical difference $p<0.05$ will be considered as statistically significant.

❖ Microscopical examination:-

Figure 1: Figure 1a and 1b Morphological characters of lab *Churna* and marketed *Churna*.

Figure 2: Microscopical characters of Triphala Churna.

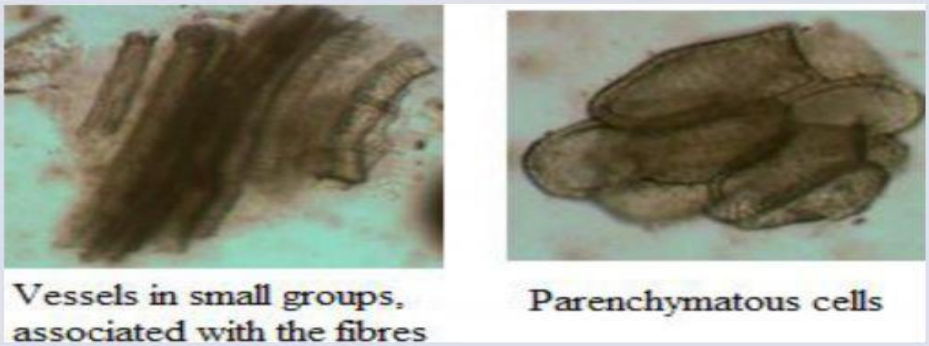


Figure 3: Microscopical characters of Amla (fr.p powder).

Ashwagandha & Triphala composition :-

Constitute	B.S	Plant part	Quantity
Amlaki	*Embellica officinalis.*	Fr.p	1 part
Bibhitaka	*Terminalia bellerica.*	Fr.p	1 part
Haritaki	*Terminalia chebula.*	Fr.p	1 part

Powder flow properties details :-

Powder flow Property	Laboratory Churna	Marketed Churna
Bulk Density*(gm/ml)	0.94±0.12	0.86±0.36
Tap Density*(gm/ml)	1.176±0.18	1.2±0.15
Hausners ratio*	1.25±0.34	1.39±0.56
Carr's index *(%)	20.06%±0.34	28.33%±0.45
Angle of Repose*	31.39±0.13	31.80±0.17

Extractive value of Churna:-

Sr. no	Sample	Water Extractive value*(%±S.D)		Alcohol Extractive value* (%±S.D)	
		%	Std Limit	%	Std Limit
1	Marketed	60.5±0.05	NLT 50	46.84±0.79	NLT 40
2	Laboratory	39.78±0.67	NLT 35	9.14±1.67	NLT 8

Loss of drying Churna:-

Sr. no	Sample	Loss on drying* (%)
1	Marketed	4.05±0.56
2	laboratory	3.25±0.24

Alma, Baheda, Harde and Ashwagandha powder show here. While laboratory as well as market formulations of mixed composition Churna shows some of similar characters. So that the locally available material can be used for Ayurvedic preparations.

Physico Chemical properties

Results of the experiment on the ash values of Churna (laboratory and market samples) and its ingredients are given it. And that reveals all ingredients having the results in agreement with those mentioned in pharmacopoeia. Our churna (laboratory and market sample) has mainly water soluble ash, Amount of acid insoluble ash suggesting the acceptable range of undesired heavy metal impurities. The results of alcohol soluble extractives and water soluble extractives of churna

(laboratory and market samples) and its ingredients are mentioned here. It appears from the results that results of extractive values of all ingredients are in agreement with those mentioned in pharmacopoeia and other references. And the results of loss on drying (laboratory and market samples) and its ingredients are mentioned . All ingredients and Churna have shown loss on drying within the Pharmacopoeial limit. This indicates that these samples contain moisture content within the acceptable range.

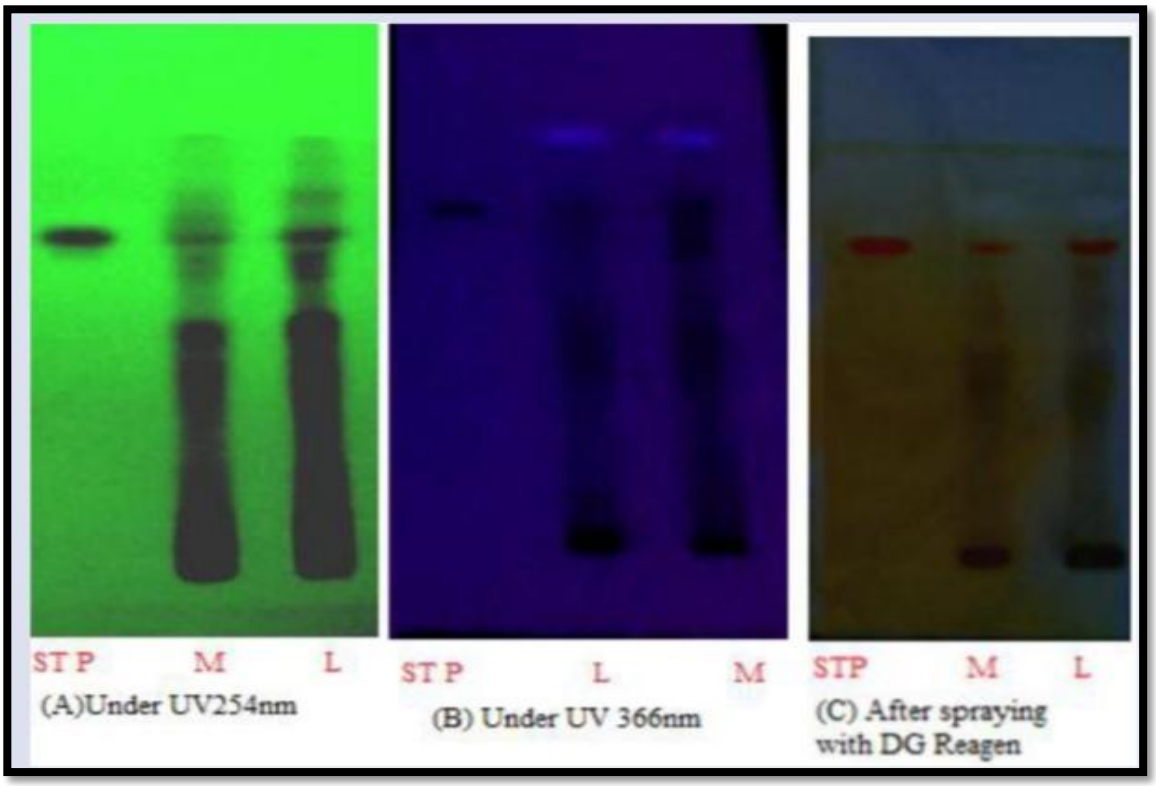
Limit test for Heavy metals of our composed churna

The color produced in the test solution is not darker than that produced in the standard solution. Therefore Churna (lab and market sample) complies with the limit of heavy metals.

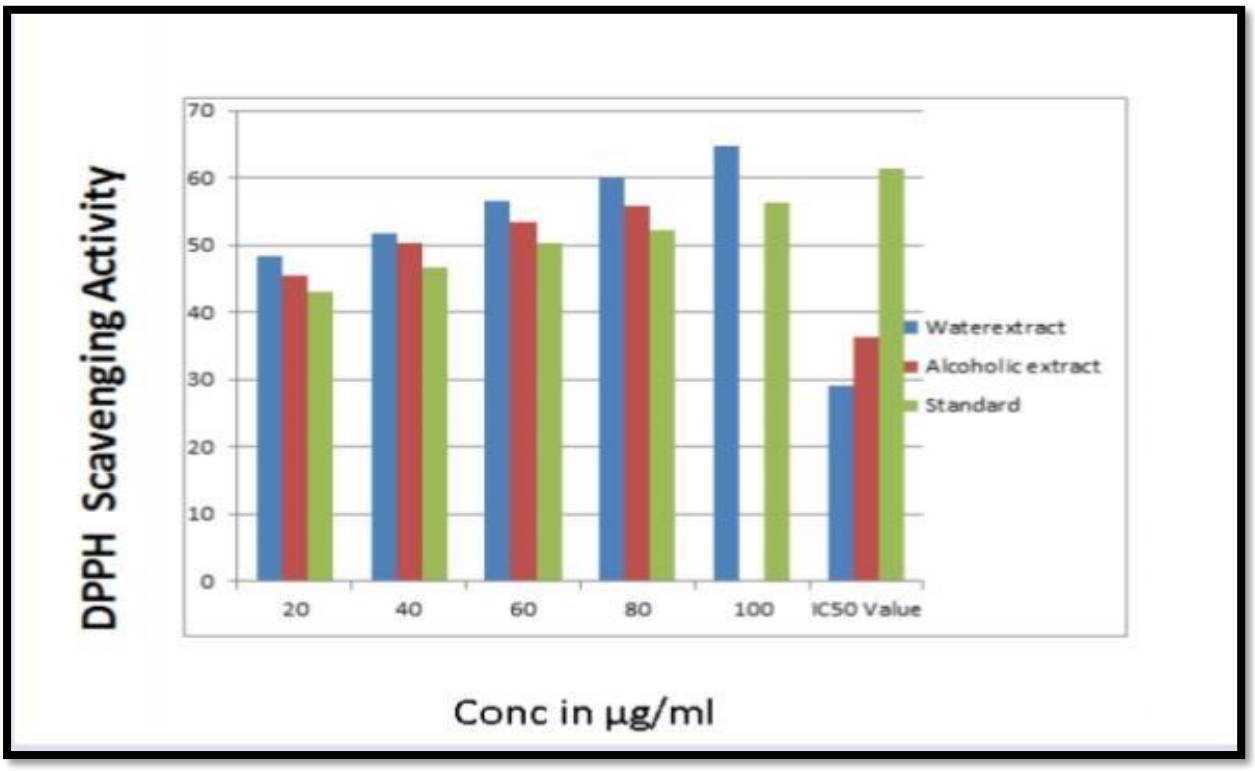
Preliminary phytochemical studies

Various Phytochemicals present in all ingredients and Churna are shown in and respectively our Churna contains Tannin, alkaloid, flavonoid and volatile oils

TLC figuring for alkaloids :-



Antioxidant activity :-



Quantitative phytochemical estimation of churna:-

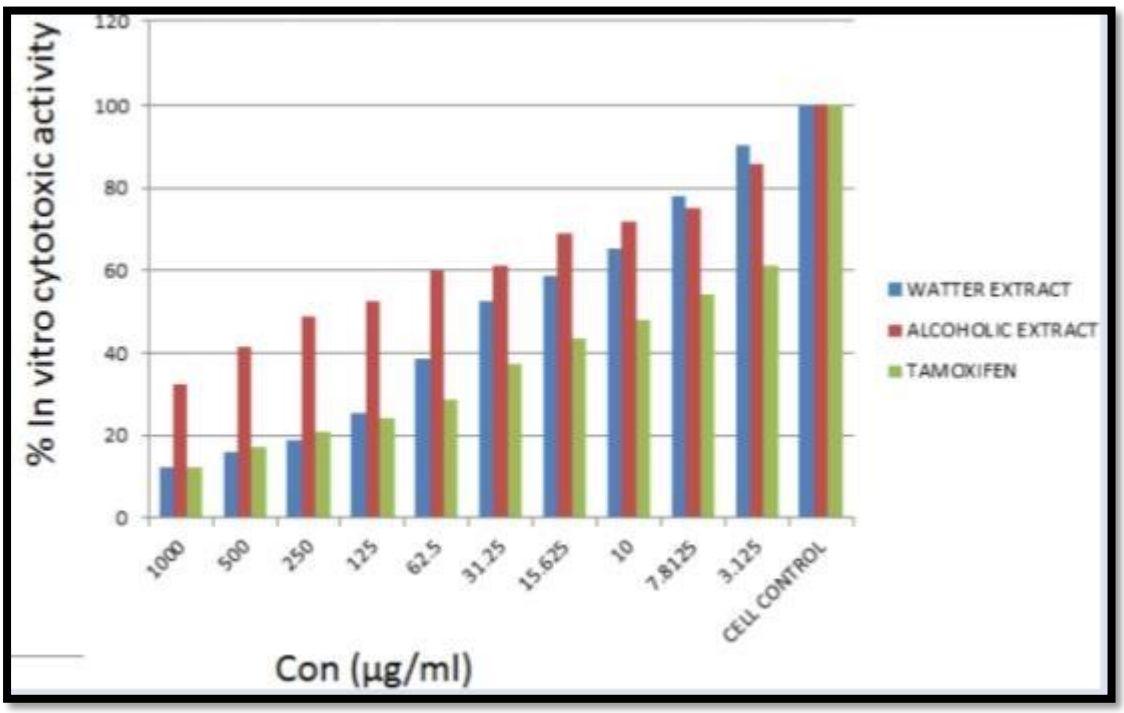
Results of the quantitative phytochemical showed here. By observing the values the our Churna having Significantly Higher Contents and this phytochemical are having good biological importance.

TLC fingerprinting of composed Churna

TLC fingerprinting for Tannins, Alkaloids

TLC fingerprinting of Churna (Lab and Market sample) for Tannins give the spot corresponds to Galiic acid with the same Rf (0.57). It gives dark blue colored spot after spraying with 5 % FeCl3 Methanol Solution. For the alkaloids Churna gives the spot corresponds to the standard piperine with the same Rf (0.83). It gives yellow colored spot after spraying with Dragndroff's Reagent. Our Churna also gives the spot at the same Rf as embolic (0.93). It gives blue fluorescence under U.V. light (366 nm).

In-vitro cytotoxic activity water and alcoholic extract :-



Phytochemicals investigation of churna :-

Sr.No	Test For	Water extract	Ethanolic
1	Phenolics and Tannins	+	+
2	Alkaloids	+	+
3	Saponins	-	-
4	Flavanoids	+	+
5	Volatile oil	+	+
6	Proteins	-	-

Qualitative phytochemicals analysis of churna:-

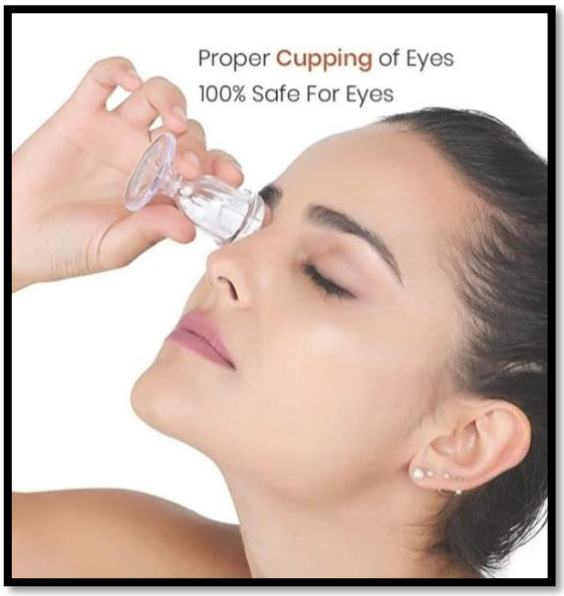
Sr no	Sample	Total Tannins	Total Phenolics	Total Alkaloid	Total Flavanoid	% Iron
1	Lab Churna	6.34±1.32	5.26±1.67	1.56±1.56	0.48±1.23	43.3±0.24
2	Market Churna	4.93±2.45	4.25±1.34	1.07±1.73	0.33±2.45	34.1±1.45

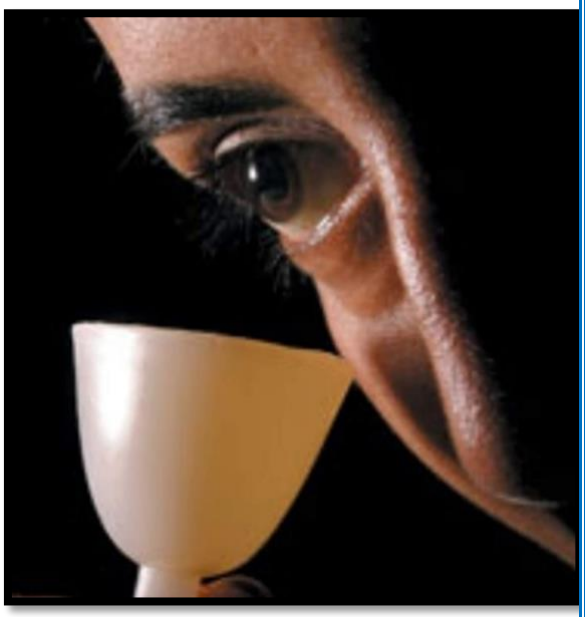
Antioxidant activity of extract of prepared churna:-

Conc in µg/ml	Water extract	Alcoholic extract	Standard
20	48.42 ± 1.03	45.4 ± 1.03	43.19 ± 1.112
40	51.78 ± 0.9	50.18 ± 0.9	46.63 ± 1.10
60	56.66 ± 0.72	53.54 ± 0.72	50.4 ± 0.83
80	59.9 ± 1.12	55.8 ± 1.12	52.29 ± 0.53
100	64.71 ± 0.69	59.35 ± 0.69	56.32 ± 0.44
IC_{50} Value	29.065	36.2789	61.46

- It’s all detail about pharmacological evaluation parameters in description etc……

❖ **Uses as other dosage form like eye wash :-**





• **How to make triphala eye wash solutions :**

Ingredients :

1 tea spoon power (Churna)

1 cup water

- Take a teaspoon organic triphala power
- Mix it hot, boiling water. The heat will help the powder dissolve and will activate the compound in triphala

How to use churna for Eye Care:

You can make a simple Triphala eyewash at home in order to strengthen your eyes and improve their health. In order to do so, you must:

- Take a teaspoon of organic Triphala powder.
- Mix it with hot, boiling water. The heat will help the powder dissolve and will activate the compounds in Triphala.
- Allow the mixture to cool until it is comfortable to touch.
- Strain the mixture through a fine cloth. This will help you remove any particles that might hurt your eyes.
- Take clean cloth or towel and dip it in the mixture.
- Le down and place the wet towel over your eyes
- Keep blinking so that your eyes can touch to the Triphala Eyewash
- Do this for at least 5 minutes
- Avoid any strain to your eyes after using the eyewash. The best time to do this is right before you sleep so that your eyes receive ample rest and relaxation.

CONCLUSION:

Form above experiment it is conclude this triphala & Ashwagandha both have good quality of amount antioxidant properties.

It also provides antidiabetic activities and good for eye sight It's also good for Glocoma, caterect, and swelling eye sight.

Ashwagandha proven anticateract activity and Triphala used good as well as Both combination can improve effect of eyesight and anticateract and compatible to each other.

So conclude that is used for improvement eye sight and cateract.

❖ RESULTS AND DISCUSSION :-

Microscopical examination

Laboratory Churna is reddish- brown in color with pungent odour and spicy, pungent taste, while market Churna is dark brown in color may be due to change in quality of raw materials the results are showed. All pass through sieve number 44 and not less than 50% pass through sieve number 85.

Powder Flow Properties

For checking their flow properties such as Bulk density, Tape density, Carr's index, Angle of repose, Housner's ratio were performed for laboratory as well as market samples. there was no significant difference between results of Lab as well as Market samples. From this values both samples we understand Churna powder having good flow properties.

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