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Research Article

Formulation and Evaluation of Poly Herbs Tablet for Hepatoprotactive Activity

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Abstract

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. The study was designed as formulation, standardization, and evaluation of polyherbal tablet prepared for hepatoprotective activity A solid pharmaceutical dosage formulation tablet was formulated by using a dry plant extract s of *Annona squamosa*, *Night Jaismine* and *Andrographic paniculata* ethanoic exracts using various excipients viz., Talc , Magnesium Stearate and MCC by direct compression method which will use for hepatoprotactive activity. The present communication also deals with the evaluation of formulated tablets (weight variation, friability, hardness and disintegration time.

Keyword : Polyherbal , Tablet, Hepatoprotective activity ,MCC, evaluation, friability.

Introduction : Medicinal plants contain inherent active ingredients to cure disease or relieve pain. The use of traditional medicines and medicinal plant in most developing countries as therapeutic agent for the maintenance of good health has been widely observed. The world health organization estimated that 80% of the population of developing countries relies on traditional medicines, mostly herbal plant drugs for their primary health care. The medicinal property of plant could be based on the anti-oxidant, antimicrobial, antipyretic effect of the phytochemicals present. Traditionally, herbs have been considered to be non-toxic and have been used for treating various problems by the general public and/or traditional medicine doctors worldwide. Although, the literature has documented several toxicity resulted from the use of herbs on many occasions, still the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine. The use of medicinal plants as raw materials in the production of drug is gaining popularity.[1,2]

Andrographis paniculata is an annual herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it has been traditionally used to treat infections and some diseases.

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Mostly the leaves and roots were used for medicinal purposes Since ancient times, *A. paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The herb has a number of purported medicinal uses, although research has found evidence of its effectiveness is limited to treatment of upper respiratory infection, ulcerative colitis and rheumatic symptoms; in particular, there is no evidence of its effectiveness in cancer treatment. According to the Mayo Clinic Book of Alternative Medicine, "A specific product (andrographis combined with Eleutherococcus senticosus) may shorten the duration and lessen the symptoms of common cold. [,3]

Annona squamosa L., the plant of Annonaceae family, also known as custard apple, is commonly found in deciduous forests, also cultivated in wild in various parts of India.

Its leaves are used as insecticidal and antispasmodic agents and are used in the treatment of rheumatism and painful spleen. The plant is reported to possess analgesic, anti-inflammatory, antipyretic, antiulcer, and antiseptic and abortifacient activities. Its use as an insecticidal agent has been investigated by several workers and various phytochemical, pharmacological, antibacterial and antiovulatory studies have already been carried out with the seed extracts. Post-cortical antifertility activity of *A. squamosa* has also been reported from studies with the seed extract [4]

Nyctanthes arbor-tristis Linn. (Oleaceae) is a traditional Indian medicinal plant, commonly called as Night Jasmine (Pavalamalli in Tamil).Extracts of the seeds, flowers and leaves possesses immunostimulant, hepatoprotective, antileishmanial, antiviral and antifungal activities in vitro (Puri *et al.*, 1994). The leaves have been used in Ayurvedic medicine and Homoeopathy for sciatica, arthritis, fevers, and as a laxative.[5]

Materials and Methods[7-14]

Collection of plant: Leaves of *Annona squamosa* and *Night Jaismine* and whole plant material of *Andrographic paniculata* were collected from ruler area of Bhopal (M.P),

Organoleptic Characters[6,7]

Plant materials were crushed in pastle and mortar to obtain a powdered form and then subsequent used for organoleptic characters. A small amount of each powdered drug was spread on a white tile and physically

examined for general appearance i.e. color, taste, texture etc.

Preparation of plant powder

The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts:

Following procedure was adopted for the preparation of ethanolic extracts from the shade dried and powdered herbs*:*

Defatting of Plant Material

All three herb materials were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction with Ethanol

The air-dried and powdered defatted material of the drugs was subjected to extraction with ethanol in a soxhlet apparatus. The extract was concentrated at 40°C with a rotary evaporator.

Determination of Percentage yield

Calculation of percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract x 100}}{\text{Weight of powdered drug taken}}$$

Phytochemical Tests

The extracts obtained by solvent extraction were subjected to various qualitative tests to detect the presence of plant constituents.

A. Alkaloids
B. Glycosides
C. Tannin
D. Flavanoid
E. Saponins
F. Carbohydrates
G. Amino acids
H. Steroids
I. Proteins
J. Carboxylic acid
K. Coumarins
L. Quinones
M. Xanthoproteins

The ethanol extract were subjected to various qualitative tests to detect the presence of plant constituents. The results have been shown in table.

Preparation of Test Solution

The test solution was prepared by taking 1 g of the extract in 25 ml of water.

A. Test for Alkaloids [7,9,10]

a) **Dragendorff's Test**: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

b) **Mayer's Test**: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

B. Test for Glycosides

a) **Legal's test**: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

b) **Keller Kiliani test**: Ethanol extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

C. Test for Tannins/Phenols

a) **Ferric Chloride test:** To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

b) **Lead acetate test:** To the sample of extract, 10 % lead acetate solution was added, white precipitate was produced.

D. Test for Flavonoids

a) **Shinoda test**: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil.

hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

E. Test of Saponins

Foam test: 1 ml of ethanolic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins

F. Test for Carbohydrates

Following tests were carried out for carbohydrates.

a) **Molisch's test:** In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

b) **Benedict's test:** In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

G. Test for Amino acid

a) **Ninhydrin's test**: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

b) **Xanthoprotein test:** To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

H. Test for Steroids and terepenoid

a) **Liebermann's Burchard reaction**: The test extract solution was dissolved in 2 ml of chloroform in a dry test tube. Now 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.

b) **Salkowsky test**: The extract of test solution dissolved in chloroform and equal volume of conc. sulphuric acid was added. Bluish red cherry, red and purple color was noted in chloroform layer, whereas acid assumes marked green fluorescence.

I. Test for Protein

a) **Biuret's test:** To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

b) **Millon's test:** The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

J. Test for Carboxylic acid

One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

K. Test for Coumarins

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

L. Test for Quinones

One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

M. Test for Xanthoproteins

One ml each of the various extracts was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

Polyherbal Tablet Formulation[11-20]

Powdering of Extract

Talc was added to convert the extracts in to powder form in *Annona squamosa* extract, *Night Jaismine* and *Andrographic paniculata* extract.

Formulation of Herbal Tablet

Tablets using extracts as active ingredients were prepared by dry granulation method. The dried powder extract and other ingredients were mixed uniformly and then the mixture was blended and granulated. The granules were compressed into tablets in an 8-station machine (**Fig. 1**).

Table No. 1: Formulation of Herbal Tablet

Ingredient	Quantity Per Tablet(mg)
Annona squamosa extract (Ethanol)	100
Night Jaismine extract (Ethanol)	100
Andrographic paniculata extract (Ethanol)	100
Talc	250
MCC	50
Magnesium Stearate	10

Fig. No. 1: Developed herbal Formulation (Tablet)

Evaluation of Polyherbal Tablet

Drug excipients compatibility study

Drug excipients compatibility study was conducted using FT-IR (Bruker alpha) Spectroscopy.

Organoleptic Properties

Shape, color and taste were determined.

Weight Variation

The USP weight variation test is run by weighing 20 tablets individually, calculating the average weight and comparing the individual tablet weights to the average. The tablets meet the USP test if no more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit. The weight variation tolerances for uncoated tablets differ depending on average tablet weight (**Table no. 2**).

For weight variation twenty tablets were weighed individually and calculated for average weight of tablet, the average weight was compared with individual tablet weight and % weight variation was determined by using following formula.

$$\% W = \{(W_o - W) / W_o\} \times 100$$

Where,

% W = Weight variation in percentage

W_o = Average weight of tablet

W = Individual weight of tablet

Hardness

The resistance of tablet to chipping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness. Several devices are used to test tablet hardness: the Monsanto tester, the Strong-Cobb tester, the Pfizer tester, the Erweka tester and the Schleuniger tester. The force is measured in kilograms and when used in production, a hardness of 4 Kg/cm^2 is considered minimum for a satisfactory tablet.

Hardness of tablet was determined by using Monsanto tablet hardness tester.

Tablet Friability

A tablet friability measurement is made by use of the Roche friabilator. This device, subjects a number of tablets to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, dropping the tablets a distance of six inches with each revolution.

Roche friabilator was used for the determination of friability. Pre-weighed 6 tablets were placed in the friabilator, which was then operated for 100 revolutions. Tablets were dusted and reweighed. The percent friability was measured using the formula;

$$\% F = \{(W_0 - W) / W_0\} \times 100$$

Where,

% F = Friability in percent

W_o = Initial weight of tablet

W = Weight of tablet after test

Disintegration Time

One tablet was placed in each of six tubes of DT apparatus. Disintegration test was performed at 37 ± 2^0 C. Disintegration time defined as time required to disintegrate and pass all fragments through the sieve (# 10)

Table No. 2: Weight Variation Tolerances for Uncoated Tablets

Average Weight of Tablets (mg)	Maximum Percentage Difference Allowed
130 mg or less	10 %
130 mg to 324 mg	7.5 %
More than 324 mg	5 %

Result and Discussion

Results of Organoleptic Evaluation

Organoleptic evaluation can be done by means of organ of sense which includes the below parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity . The organoleptic investigations (color, odour and taste, and texture) were performed.

From the results obtained in table no.3 it is clear that the *Annona squamosa* shows the brown colour, aromatic or characteristic odour, characteristic taste and Yellowish and foliaceous texture, *Night Jaismine* and *Andrographic paniculata* also shows same oraganoleptic characters except difference in their colour.

Determination of Percentage yield

Calculation of percentage yield

The crude extracts so obtained after the soxhlet extraction process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant in particular solvent was used. The yield of extracts obtained using ethanol as solvents are depicted in the table no. 4.

Results of phytochemical Evaluation

A small portion of the dried extracts were subjected to the phytochemical test using (**Kadam *et al*., 2013)** methods to test for carbohydrate, alkaloids, steroid, glycosides, tannins, saponins, flavonoids , protein, starch & amino acid separately for extracts of all samples. Small amount of each extract is suitably centrifuged into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table no 5-7.

From the results obtained it is clear that the *Andrographic paniculata*, *Night Jaismine and Annona squamosa* ethanolic extract shows the presence of Protein, Phenol Amino Acid, Steroid and Flavonoids. The phytochemical analysis of ethanolic extract of *Night Jaismine and Annona squamosa* plant indicates the presence of Carbohydrate, Protein, Glycoside, Flavonoid, Alkaloid and Tannins. Flavonoids and Phenol are the phytochemicals that are present in all the extracts. Ethanolic extracts of *Andrographic paniculata*, *Night Jaismine and Annona squamosa* were found highly rich in flavanoids and Phenols, although saponins are totally absent in them. Flavonoids and Phenols are the only which are present in all the extracts under evaluation.

Drug excipients compatibility study

Results of FT-IR spectra clearly indicate that the functionality present in polyherbal preparation remains unchanged in polyherbal tablets formulation. The results indicate that there is no interaction between polyherbal extract and excipients used in polyherbal tablet formulation.

Evaluation of developed herbal formulation (tablet) organoleptic properties

The prepared herbal tablets were evaluated for their organoleptic properties and the results were shown in Table no. 8

Weight Variation

Twenty tablets were selected randomly and evaluated for weight variation and the results were shown in Table no. 9

Hardness

The tablets were prepared by applying maximum force of compression and the average hardness of the tablet was found to be in the range of 4 to 4.5 kg/cm^2. Table no. 9.

Friability

Six tablets were randomly selected and evaluated for friability.

Disintegration Time

Disintegration test was performed at 37 ± 2^{0}C in DT apparatus and the average disintegration time of the tablet was found to be 16 min.

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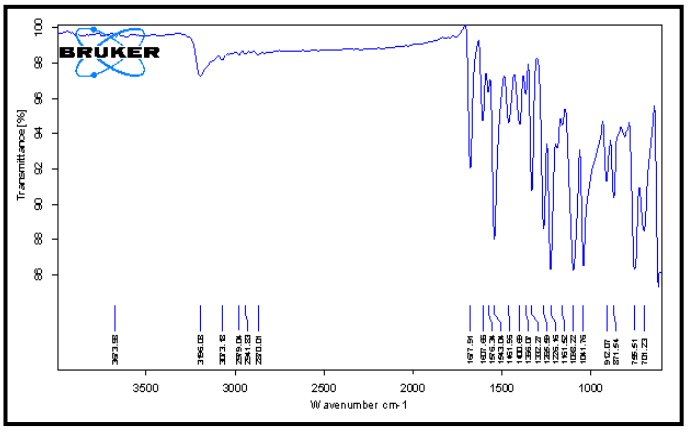

Fig. No. 2: FT-IR spectra of Polyherbal Extract

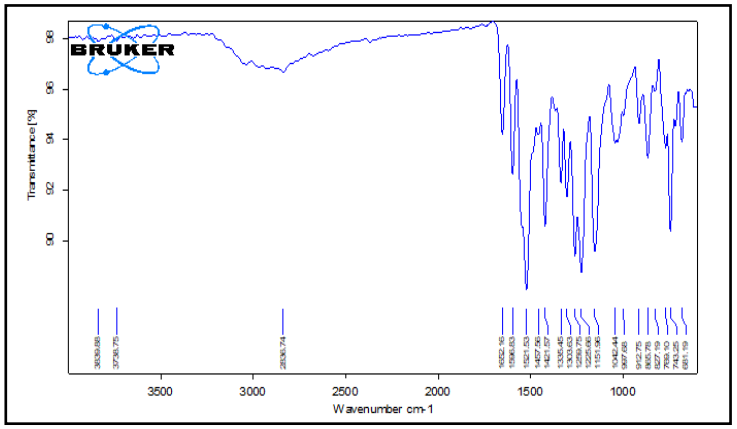

Fig. No. 3: FT-IR spectra of Polyherbal Tablets+ Excipients

Table No. 3: Organoleptic Characters of Chosen Drugs

Drug	Color	Odour	Taste	Texture
Annona squamosa	**Green**	**Aromatic or Characteristic**	**Characteristic**	**Yellowish and foliaceous**
Night Jaismine	**Dark green**	**Characteristic**	**Bitter**	**Yellowish and foliaceous**

Drug	Color	Odour	Taste	Texture
Annona squamosa	**Green**	**Aromatic or Characteristic**	**Characteristic**	**Yellowish and foliaceous**
Andrographic paniculata	**Dark brown**	**Characteristic**	**Bitter**	**Yellowish Black and foliaceous**

Table No. 4: Results of Percentage yield of *Annona squamosa, Night Jaismine* and *Andrographic paniculata*

S. No.	Extracts	Percentage yield (%)		
		Annona squamosa	***Night Jaismine***	***Andrographic paniculata***
1.	Chloroform	2.58	3.54	3.78
2.	Ethyl acetate	6.54	6.44	5.7
3.	Ethanolic	11.21	12.23	13.45
4.	Aqueous	8.98	8.74	9.57

Table No. 5: Phytochemical Evaluation of *Annona squamosa* extracts

Chemical Tests	Chloroform	Ethyl acetate	Ethanolic	Aqueous
Alkaloids				
Mayer's reagent	+	+	+	+
Dragendorff's reagent	+	+	+	+
Glycosides				
Legal's test	-	-	-	+
Keller-Kiliani	-	-	-	+
Phenols/Tannins				
Ferric chloride	-	-	+	+
Lead acetate test	-	-	+	+
Flavonoids				

Shinoda test	+	+	+	+
Saponins				
Foam test	-	-	-	+
Carbohydrates				
Molish test	-	-	-	+
Benedict's test	-	-	-	-
Amino acids				
Ninhydrin Test	-	-	+	-
Steroids & Terpenoids				
Lieberman Burchard Test	-	-	+	+
Salkowski test	-	-	+	+
Protein				
Biuret test	-	-	+	-
Million's test	-	-	+	-
Carboxylic acid				
Sodium bicarbonate test				
Coumarins	-	-	+	+
Quinones	-	-	-	-
Xanthoproteins	-	-	-	-

(+) Indicates 'Presence'; (-) Indicates 'Absence

Table No. 6: Phytochemical Evaluation of *Night Jaismine* extracts

Chemical Tests	Chloroform	Ethyl acetate	Ethanolic	Aqueous
Alkaloids				
Mayer's reagent	+	+	-	+
Dragendorff's reagent	-	+	+	-
Glycosides				

Legal's test	-	-	+	+
Keller-Kiliani	-	-	+	+
Phenols/Tannins				
Ferric chloride	+	+	+	+
Lead acetate test	-	-	+	+
Flavonoids				
Shinoda test	-	-	+	-
Saponins				
Foam test	-	-	-	-
Carbohydrates				
Molish test	-	+	+	-
Benedict's test	-	-	+	+
Amino acids				
Ninhydrin Test	-	+	+	+
***Steroids* & Terpenoids**				
Lieberman Burchard Test	-	-	+	+
Salkowski test	-	-	+	+
Protein				
Biuret test	-	-	+	-
Million's test	-	+	+	-
Carboxylic acid				
Sodium bicarbonate test	-	-	-	-
Coumarins	-	-	-	-
Quinones	-	-	-	-
Xanthoproteins	-	-	-	-

Table No. 7: Phytochemical Evaluation of *Andrographic paniculata* extracts

Chemical Tests	Chloroform	Ethyl acetate	Ethanolic	Aqueous
Alkaloids				
Mayer's reagent	-	-	+	+
Dragendorff's reagent	-	-	+	-
Glycosides				
Legal's test	-	-	+	+
Keller-Kiliani	-	-	+	-
Phenols/Tannins				
Ferric chloride	+	-	+	-
Lead acetate test	+	-	+	+
Flavonoids				
Shinoda test	+	-	+	+
Saponins				
Foam test	-	-	+	+
Carbohydrates				
Molish test	-	-	+	+
Benedict's test	-	-	+	+
Amino acids				
Ninhydrin Test			+	
***Steroids* & Terpenoids**				
Lieberman Burchard Test	+	-	-	-
Salkowski test	-	-	-	-
Protein				
Biuret test	-	-	+	+
Million's test	-	-	+	-

Carboxylic acid				
Sodium bicarbonate test	-	-	-	
Coumarins	-	-	-	
Quinones	-	-	-	-
Xanthoproteins	-	-	+	+

Table No. 8: Organoleptic Properties of Developed Herbal Formulation

Organoleptic Parameter	Results
Shape	Round
Color	Light Brown
Taste	Characteristics

Table No. 9: Hardness of Developed Herbal Formulation

Tablet No.	Hardness (kg/cm^2)	Weight Variation
1.	4.0	Passes
2.	4.5	Passes
3.	4.3	Passes
4.	4.0	Passes
5.	4.5	Passes
6.	4.2	Passes

Table No. 10 : Characteristics of Developed Herbal Formulation

S. No.	Evaluation Parameters	Results	Remark
1.	Weight variation	None of the tablets out of the limit	Within Limit
2.	Average hardness	4.25 kg/cm^2	-
3.	Average % friability	0.54	Within Limit
4.	Average disintegration Time	16 min.	-
5.	Friability	0.562%	Pass