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PREPARATION AND STANDARDIZATION OF POLYHERBAL EXTRACTFOR ANTIOXIDANT ACTIVITY

D. Venkatanarayana*, V. Sreedhar, B. Chakrapani, Sk. Shabanaazmi, D. Shakila, B. Saipriyanka, S. Shakeela, T. Sainath

Balaji College of Pharmacy, Anantapuramu, Andhra Pradesh, India.

*****Corresponding author E-mail: **venkatanarayana1978@gmail.com**

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ABSTRACT

The number one purpose of the study is to preparation and standardization of polyherbal (*Glycyrrhiza glabra*, *Withania somnifera* and *Emblica officinalis*) aggregate for anti-oxidant activity. Polyherbal combination became prepared with the useful resource of mixing them in equal ratio. This combination was extracted with ethanol. The PHE has been standardized on the basis of morphological, physical and physicochemical properties. The antioxidant activities had been evaluated by using DPPH and power reducing method. The consequences acquired from morphological, physical, physicochemical parameters evaluation shows that the contents of preparation present are within permissible limits. Antioxidant activities of the Polyherbal aggregate extract become additionally assessed by means of DPPH assay, and reducing powder assay. They showed antioxidant effect as that of the ascorbic acid. The antioxidant properties were due to the presence of promising phytoconstituents in Polyherbal mixture.

INTRODUCTION

Plants have been used worldwide in conventional drug treatments for the treatment of several illnesses and it is expected that even these days approximately 65-75% of the world's population depend best on medicinal flora as their number one deliver of medicines. India is one of the few worldwide places inside the global which has specific wealth of medicinal plant life and considerable conventional knowledge of use of natural medicine for treatment of numerous illnesses (1). The mixture of various herbs (polyherbal) in a selected ratio will supply a applicable therapeutic effect because the powerful phytochemical elements of individual plant life are insufficient to achieve the beneficial effect. The polyherbal components consists of two or greater herbs with special phytoconstituents owning similar or distinct therapeutic capability have been collectively generating appropriate outcomes at some stage in the control of human illnesses. The recognition of the polyherbal additives is first-rate because of their big therapeutic variety i.e., effective at a low dose and secure at immoderate dose, even though produces fewer fact effects whilst misused (2-7). Antioxidants play a main role in helping to protect our body from the formation of free radicals and save you or dispose of the superiority of lipid peroxidation. In our frame, oxidants and free radicals, which can be formed from triplet oxygen, water and unsaturated lipid molecules, can prompted oxidative pressure in tissue of lungs, heart and cardiovascular system, kidneys, liver,

gastrointestinal tract, blood, eye, skin, muscle and brain. Therefore, free radical is to be predominant precursor for the improvement of several degenerative sicknesses, along with cancer, atheroschlerosis, gastric ulser, diabetic, and others. An intensive observe has been carried out to evaluate antioxidant belongings of compounds originated from terrestrial plant resources. Phenolics plant, specially phenolic acids, tannins and flavanoids are recognised to be as strong antioxidants (8).

MATERIALS AND METHODS

Selection of plant material: All the three plants had been selected on the idea of their antioxidant activity previously studied the use of DPPH and reducing power technique.

Collection of crude drugs: Rhizomes of *Glycyrrhiza glabra* roots of *Withania somnifera* and fruits of *Emblica officinalis* have been purchased from the local shops.

Preparation of polyherbal powder: Polyherbal powder changed into made by taking equal share of each powder natural drugs. All of the procured individual crude drug cloth is dried in color and wiped smooth with the resource of hand sorting. The individual drugs are then pulverized the usage of a mill and passed through mesh no.40 one after the other and automatically mixed the ones powder in same ratio. similarly, it modified into packed in tightly closed box and stored in a cool and dry place.

Preparation of polyherbal extract (PHE): Polyherbal powder becomes prepared by using soxhlet extraction approach using ethanol and a decoction comes to be prepared. Decoction turned into filtered via clear out fabric to gain ethanol extract, focused under vacuum the usage of rotatory evaporator at the way to do away with the ethanol content material and gain the extract in dry stable form. Percentage yield = Weight of extract/ Weight of Powder drug x 100.

Morphology of PHE: The morphology or macroscopical parameters like colour, odour, and taste had been studied for PHE with the aid of sensory organs.

Physicochemical parameters of PHE : The diverse Physicochemical parameters like extractive values, ash values and loss on drying had been done with polyherbal extract(9).

Determination of total ash: Appropriately weighed 1gm air dried PHE was placed in a formerly ignited and tarred crucible. The material was spread in an even layer and ignited it by means of the usage of step by step increasing the warmth to 400- 500ºC until it was white, indicating the absence of carbon. It changed into cooled in desiccator and weighed. If carbon free ash can't be received in this way, the crucible became cooled and residue was moisten with 2ml water and dried on a water bath, ignited to steady weight. The residue become allowed to cool in desiccators for 30mins and it was weighed right now. the percentage of overall ash was calculated with reference to air-dried plant material.

Acid insoluble ash: The regarded quantity of the ash was taken inside the crucible containing 25ml HCl. The insoluble matter was collected on an ash less filter-paper and washed with hot water till the filtrate become impartial. The filter paper ignited in a crucible for 15mins at a temperature not exceeding 450^0C. The consequently percent of acid insoluble ash was calculated with regards to air dried plant material.

Water soluble ash: The ash received as above become taken in crucible containing 25ml of water and boiled for 5mins. The insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper ignited in a crucible for 15mins at a temperature no longer exceeding 450^0C. The percentage of water soluble ash was calculated with reference to air dried plant material.

Determination of extractive matter

Alcohol soluble extractive: Appropriately weighed 2gmPolyherbal extract powder material became positioned in a glass

stoppered conical flask and macerated with 100ml alcohol (ethanol) for 24hr. It became shaken often for the first 6hr and allowed to stand for 18hr. It was filtered rapidly taking care not to lose any solvent and then transferred 25ml of the filtrate to tarred flat-bottomed dish and evaporated to dryness on water bath. It was dried at 105°C for 6hr, cooled in a desiccator for 30min and weighed at once. As a consequence percent of alcohol soluble extractive value was calculated on the subject of air-dried drug.

Water soluble extractive: Accurately weighed 2gmPolyherbal extract powder material became located in a glass-stoppered conical flask and macerated with 100ml water for 24hr. It was shaken regularly for the primary 6hr and allowed to stand for 18hr. It was filtered hastily taking care not to lose any solvent after which transferred 25ml of the filtrate to tarred flat-bottomed dish and evaporated to dryness on water tub. It became dried at 105°C for 6hr, cooled in a desiccators for 30min and weighed without delay. Thus percentage of water soluble extractive value was calculated on the subject of air-dried drug.

Loss on drying: Correctly weighed 2g Polyherbal extract powder material become located in a tarred evaporating dish. It became dried at 105ºC for 5hrs, after placing the drug into the tarred evaporating dish and weighed. The drying and weighing turned into persevered at one hour interval until constant weight. Percent moisture content material became calculated on the idea of sample taken.

Physical characteristicsof PHE(10-12)

Bulk density and Tapped density:

The term bulk density refers to a measure used to describe a packing of particles. The equation for determining bulk density

Bulk density = Mass of powder/Bulk volume

The volume of the packing may be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device. 100gm of weighed extract powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped. Until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.

Tapped density: It is defined as the volume occupied by same mass of powderafter standard tapping. Tapped density = Mass of powder/Tapped volume

Carr's index: It is related to flow rate particle size andcohesiveness by knowing the Carr's index can be predicted by flow characteristic of powder.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} X100$$

Hausner's ratio: It is defined as ratio of the tapped density to the bulk density.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

A hausner ratio lower than 1.25 indicates a powder is free flowing whereas higher than 1.25 indicates poor flow property of powder.

Angle of repose: Angle of Repose has been used as an indirect method of quantifying powder flowability; due to its relationship with interparticle cohesion. As a general guide, powders with angle of repose more than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel.

$\tan \alpha = H/R$ or $\alpha = \arctan H/R$

Where H= height of the pile, cm

R= radius of the base of pile,cm

Preliminary Phytochemical Screening: (13-14): PHE powder was analyzed for the presence of numerous elements like alkaloids, carbohydrates, glycosides, saponins, phytosterols and steroids,

flavonoids, tannins and phenolic compounds and proteins.

Anti-oxidant activity

Determination of DPPH Radical Scavenging Activity (15-16):

The PHE was treated with 2, 2 Diphenyl-1-picryl hydrazyl radical to estimate its free radical scavenging activity using UV-Spectrometry at 517nm. Ethanol was used for the preparation of DPPH solution. For the preparation of stock solution PHE was mixed with ethanol. The 2ml, 4ml, 6ml, 8ml and 10ml of stock solution become taken in 5 test tubes and identical solvent become used to make up 10ml of solution in each test tube by serial dilution technique. The concentration of test tubes was 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml. Further, freshly prepared 0.004% w/v of DPPH solution introduced to each test tube then spectrophotometer reading becomes taken after ten mins of the addition at 517nm. Ascorbic acid was used as standard drug and distilled water was used for the preparation of stock solution. The control sample was prepared without extract and 95% ethanol was used as blank. The activity is expressed as percentage removal by the DPPH free radical.

$$\% \text{ DPPH radical scavenging} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

Reducing Power Method (17-20)

The One ml of PHE was combined with 2.5 ml phosphate buffer and 2.5 ml Potassium Ferric-cyanide. The combination was incubated at 50°C for 20 minutes. 2.5 ml of Trichloroaceticacid was added and the combination was subjected to centrifuge action at 3000 rpm for 10 min. 2.5 ml of the supernatant solution was collected and combined with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ and using UV-visible spectrophotometer, absorbance calculated at 700nm. The standard and blank used were Ascorbic acid and phosphate buffer respectively.

RESULTS AND DISCUSSION

Preparation of Polyherbal extract:

Table No. 1Percentage yield of Polyherbal extract

Extract	**Yield %w/w**
PHE	16.2

Ethanol extract of Polyherbal powder had shown yield 16.2 %w/w

Property	PHE
Nature	Crystalline powder
Colour	Light yellow
Odour	Characterstic
Taste	Characterstic

Organoleptic parameters of PHE

The organoleptic evaluation revealed that Polyherbal extract is a powder with nature, colour, odour and and taste.

Physicochemical properties of PHE: Polyherbal extract powder subjected to various physiochemical parameters like, total ash,water soluble ash, acid insoluble ash,water soluble extract, alcohol soluble extract, moisture content for confirming their pharmacognostical profile.

Table No. 3 Physical constants

Parameters	PHE (%w/w)
Total ash	3.3
Water soluble ash	1.22
Acid insoluble ash	0.81
Water soluble	5.5
Alcohol soluble	6.7
Loss on drying	3.5

Result specified that total ash, water soluble ash, acid insoluble ash, water soluble extract , alcohol soluble extract, moisture content was 3.3, 1.22, 0.81, 5.5, 6.7 and 3.5 respectively.

Flow property: Flow properties of the PHE powder was checked using bulk density, tapped density, hausner's ratio,carr's index and angle of repose as per the standard procedure and results were described in Table no 4.

S.No.	Parameters	PHE
1	Bulk density (g/cc)	0.43
2.	Tapped density (g/cc)	0.77
3.	Hausner's ratio	1.79
4	Carr's index	41.30
5	Angle of repose	25.2

Table no: 4 Flow properties of PHE

Phytoconsituents	PHE
Flavanoids	+
Alkaloids	+
Tannins	+
Saponins	+
Steroids	+
Carbohydrates	+
Aminoacids	+

Table No. 5- Preliminary Phytochemical screening ofpolyherbal extract,

+ = Positive

Bulk density, tapped density, hausner's ratio, carr's index and angle of repose of PHE were 0.43 (g/cc), 0.77 (g/cc), 1.79, 41.30 and 25.2 respectively.

Phytochemical screening: Ethanol extract of polyherbal powder was subjected for the various phytochemical tests like flavonoids, alkaloid, tannins, saponin, steroides, carbohydrate and amino acids. Results are given in Table 5. Result indicated that alcoholic extract of polyherbal powder had shown the presence of flavonoid, alkaloids, tannins, Saponins, Steroids,carbohydrates and amino acid.

Anti-oxidant activity

Diphenyl picryl hydrazyl method: The DPPH scavenging effect increased with the increasing concentrations of PHE as compared to standard ascorbic acid.

S. No.	Concentration (µg/ml)	% scavenging DPPH of Ascorbic acid	%scavenging DPPH of PHE
1	20µg/ml	37.45	32.05
2	40µg/ml	51.30	49.22
3	60µg/ml	62.31	55.63
4	80µg/ml	76.11	64.31
5	100µg/ml	92.47	79.27

Table No. 6: Effect of PHE on DPPH method

S. No.	Concentration (µg/ml)	Absorbance of PHE	Absorbance of Ascorbic acid
1	20	0.20	0.39
2	40	0.29	0.45
3	60	0.55	0.66
4	80	0.76	0.80
5	100	0.87	0.95

Table No. 7 Effect of PHE on reducing power assay

PHE has shown good Reducing powder activity that was comparable with ascorbic acid. Increased absorbance ofthe reaction mixture indicated the increased reducing powder and the highest reducing poweractivity of PHE were found to be 87% inhibition at 100 µg/mlconcentration as compared to ascorbic acid.

CONCLUSION

The present study also showed that PHE have significant antioxidant activity. The beneficial effects of PHE in oxidative disorders may be attributed to the presence of multiple ingredients with multiple modes of actions. The results from this study rationalize the medicinal use of PHE in oxidative disorders.

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