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Assessment of neuroprotective potential of a polyherbal formulation having anti Parkinson effects

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Abstract
The main objective of this study was to identify the antioxidant potential of a Polyherbal formulation. It involves preparation of a poly-herbal formulation by preparing different polar solvent extract of the four plants namely, *Mucuna Pruriens* (MP), *Bacopa monnieri* (BM) & *Withania somnifera* (WO), were extracted using Soxhlet Apparatus. Extracts obtained by different solvents (ethanol, ethyl acetate, chloroform, and n-hexane) were tested for total antioxidant capacity, DPPH against Scavenging activity, and total polyphenol and flavonoid contents. The antioxidant potential (DPPH Scavenging activity) of the above formulation was compared with other similar marketed formulations. The screening done by using DPPH scavenging activity showed that the free radical scavenging effect of Polyherbal formulation (PHF) at concentration 100 µg/ml (i.e. 92. 43%) reveals maximum percentage inhibition of free radicals. The effects mentioned at concentration 25 µg/ml and 50 µg/ml were found to be more antioxidant efficacy than reference standard drug. Finally the results concluded that, all the used solvents are able to extract compounds having high antioxidant activity except as regards n- hexane, when compared to ascorbic acid.

Keywords: *Mucuna Pruriens* (MP), *Bacopa monnieri* (BM) & *Withania somnifera* (WO), polyherbal formulation

Introduction
Parkinson's disease (PD) is a common neurodegenerative disease characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. The main pathological change in PD is progressive loss of dopaminergic neurons in the substantia nigra of the midbrain, though the cause of cell death is unknown. It is characterised by motor abnormalities including tremor, muscle rigidity, paucity of voluntary movements and postural instability. The main neuropathological feature is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and their projections to the striata (Dauer and Przedborski, 2004) [35],
The main causes of the neuronal degeneration in these diseases along with environmental factors, genetic mutations and brain aging, are several cellular and molecular events such as increase in oxidative stress, impaired mitochondrial functions, deposition of aggregated proteins, inflammatory response, activation of neuronal apoptosis, altered cell signalling and gene expression. (Jellinger, 2001; Parihar *et al*., 2008) [37, 31] Substantial evidence indicates that polyherbal formulations containing antioxidants are of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants in form polyherbal formulations, rather than single entities, may be more effective over the long term. (DP Shelke, GR Shendarkar 2017) [4]. Neuroprotective treatment does not directly address the etiology of PD, but intervention in some intermediate links in pathogenesis can delay the development of disease. Traditional medicines have shown potential clinical efficacy in attenuating the progression of PD. Growing evidence indicates that some Chinese herbs contain neuroprotective compounds, such as resveratrol, curcumin, or ginsenoside, green tea polyphenols or catechins, triptolide, etc. (Virmani A *et al* 2013, Lee WH *et al* 2013, Kim HJ *et al* 2013, Sun AY *et al* 2010, Chen LW *et al* 2007) [22, 23, 24, 25, 26]
The present paper aimed to determine the neuroprotective activity of polyherbal formulation containing extract of the four plants namely, *Mucuna Pruriens* (MP), *Bacopa monnieri* (BM), *Withania somnifera* (WO) and *Emblica officinalis* (EO) as component mixture. These extracts were used further for preparation of poly-herbal formulation. Extracts obtained by different solvents (ethanol, ethyl acetate, chloroform, and n-hexane) were tested for total antioxidant capacity, total reducing power, scavenging activity against DPPH radical, and total polyphenol and flavonoid contents.

Material and method
Plant collection & Authentication
The vegetative portion of plants i.e. Aqueous extract of the four plants namely, *Mucuna Pruriens* (MP), *Bacopa monnieri* (BM), *Withania somnifera* (WO) and *Emblica officinalis* (EO), were procured from local market of Lucknow. Herbarium sheets were prepared and authenticated by Taxonomist. Specimen no: NPC/PhD./Herbarium 2018/ H-09.

Plant profile
JALBRAHMI (*Bacopa monnieri*)
Botanical classification
Kingdom: Plantae
Division: Magnoliophyta
Order: Lamiales
Family: Scrophuariaceae
Genus: *Bacopa*
Species: *monnieri*

Fig 1: *Bacopa monnieri*

Ashwagandha (*Withania somnifera*)
Botanical classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Solanales
Family: Solanaceae
Genus: *Withania*
Species: *somnifera*

Fig 2: *Withania somnifera*

***Mucuna pruriens* (seeds)**
Botanical classification
Order: Fabales
Family: Fabaceae (Leguminosae)
Subfamily: Faboideae
Genus: Mucuna
Species: *Mucuna pruriens* (L.) DC.

Fig.3: *Mucuna pruriens*

AMLA *(Embellica officinalis*)
Botanical classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Malpighales
Family: Euphorbiaceae
Genus: *Emblica*
Species: *officinalis*

Fig 4: *Embellica officinalis*

Physical evaluation of powder
The collected part of plant were dried by using shade drying method for complete removal of moisture. It was further powdered and finally passed through sieve no 40 # size to obtain uniform sized powdered particles. Physical evaluation of individual powder was done for following parameters. The results are shown in table no.01.

1. Ash value
2. Extractive value
3. LOD
4. pH
5. Solubility

Preparation of Polyherbal Formulation (PHF)
Phytochemical evaluation of PHF Test for identification:
The PHF was tested to evaluate the presence of various phytoconstituents in it by performing test for identification of alkaloids, glycosides, tannin, flavonoids, amino acid, carbohydrates, steroids etc. The PHF was further tested for determination of total Phenolic content and total flavonoid content in it by using following methods.

Total Phenolic content
The total phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot (1ml) of PHF or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% NaHCo3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer.

Total flavonoids content
Total flavonoid content was measured by the aluminium

chloride colorimetric assay. An aliquot (1ml) of PHF or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm.

Antioxidant activity

DPPH assay (2, 2-Dipheny l-1-Picrylhydrazyl)

The free radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (50mg/ml) in methanol was used as reference standard.

Principle

2,2 Diphenyl 1,1- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

Method

Different concentrations (25, 50 & 100 µg/ml) of PHF were made with methanol and 1ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 30 min. After 30 min, the absorbances of the mixture were measured at 517 nm. 1ml of DPPH and 1 ml of Methanol was taken as control.

Where,

RSA is the Radical Scavenging Activity.

Abs control is the absorbance of DPPH + Methanol.

Abs sample is the absorbance of DPPH + PHF.

Results

Powdered drug evaluated for different physical parameters showed the following results.

Table 1: Physical Parameter

Sr. no	Physical parameter	Drug name & Obtained value (%w/w)			
		Withania somnifera	***Emblica officinalis***	***Bacopa moneri***	***Mucuna pruriens***
1	Ash Value	7.2%	7.7%	10.5%	9%
2	Water soluble ash	3.5%	3%	3.5%	5.5%
3	Acid insoluble ash	3 %	2.5 %	8 %	3.5 %
4	Extractive value	8 %	9 %	52 %	12 %
5	LOD	7.5 %	7.9 %	7 %	7 %
6	pH	7.45			
7	Solubility	Ethyl acetate, Chloroform, Ethanol			

The PHF was screened for phytochemical evaluation showed presence of following phytoconstituents in it.

Table 2: Phytochemical analysis of Polyherbal formulation

Sr. no	Test Name	Observation
	Flavonoids	
1	Shinoda test	+
	Alkaline test	+
	NH_4OH test	+
	Glycosides	
2	Keller Killani test	+
	Modified Borntragers test	+
	Steroids	
3	Liebermann-Buchard test	+
	Salwoski test	+
	Alkaloids	
4	Wagners test	+
	Hagers test	+
	Mayers test	-
	Carohydrates	
5	Molish test	+
	Benedicts test	+
	Barfoed test	+
	Proteins	
6	Million test	+
	Biuret test	-
	Xanthoprotein test	-
	Amino acid	
7	Ninhydrin test	-
	Tyrosine test	+
	Tryptophan test	+
	Tannins & Phenolic Compound	
8	$FeCl_3$	+
	Dil. HNO_3	+
	Lead acetate	-

Table 3: Total Phenolic Content

Concn of PHF (µg/ml)	Absorbance
Polyherbal formulation (10 µg/ml)	1.254

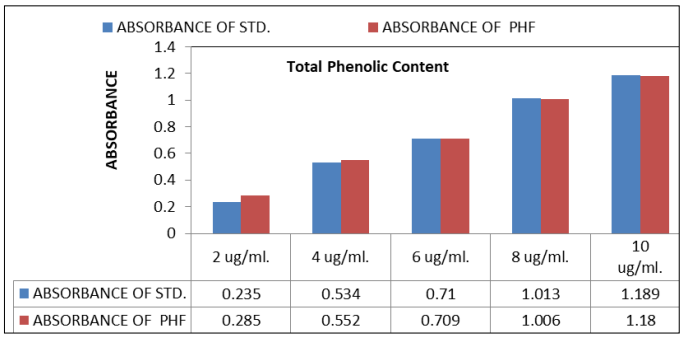

Fig 5: Total Phenolic content

Table 4: Total Flavonoid content

S. No.	Concentration (ug/ml.)	Absorbance of STD.	Absorbance of PHF
1	2 ug/ml.	0.235	0.285
2	4 ug/ml.	0.534	0.552
3	6 ug/ml.	0.710	0.709
4	8 ug/ml.	1.013	1.006
5	10 ug/ml.	1.189	1.180

Concn of PHF (µg/ml)	Absorbance
Polyherbal formulation (150 µg/ml)	1.124

S. No.	Concentration (ug/ml.)	Absorbance Of STD.	Absorbance OF PHF
1	50 ug/ml.	0.491	0.385
2	100 ug/ml.	0.791	0.754
3	150 ug/ml.	1.2	1.101
4	200 ug/ml.	1.505	1.452
5	250 ug/ml.	1.834	1.698

Conclusion and Discussion

The antioxidant potential (DPPH Scavenging activity) of the above formulation was compared with other similar marketed formulations. The screening done by using DPPH scavenging activity showed that the free radical scavenging effect of Polyherbal formulation (PHF) at concentration 100 µg/ml (i.e. 92. 43%) reveals maximum percentage inhibition of free radicals. The effects mentioned at concentration 25 µg/ml and 50 µg/ml were found to be more antioxidant efficacy than reference standard drug. Finally the results concluded that, All the used solvents are able to extract compounds having high antioxidant activity except as regards n- hexane, when compared to ascorbic acid.

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