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Research Article

Formulation Development, Quality Evaluation and *In Vitro* Antioxidant Activity of a Polyherboceutical Composition

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ABSTRACT

Polyherbal preparations have been used for health management for decades. Allopathic dosage forms are prominent due to their ease of administration and stability, but polyherbal formulations are also pharmacologically effective and show useful therapeutic activity. As a result, extracts of a six potent herbs with anti-oxidant and anti-hypertensive properties were chosen and formulated in a capsule dosage form. This study aims to investigate the anti-oxidant activity of a polyherboceutical formulation. A polyherboceutical formulation were prepared using six ingredients, and quality control parameters were performed, including organoleptic, physio-chemical, qualitative, and quantitative estimation of secondary metabolites and microbial load. The formulation was possessed a good anti-oxidant activity with 7.87 mg/mL for IC_{50} and 0.5 of absorbance on 8.6 mg/mL concentration found with DPPH free radical scavenging activity and ferric-ion reducing activity simultaneously.

Introduction

Extracts of plant materials are known to be more effective when compared with powdered plant materials as a whole.[1] Different procedures have been adopted for extract preparation in order to obtain the maximum yield of therapeutically active constituents. Extracts are more advantageous in terms of estimation of quantitative phytoconstituents rather than herbs. Hot percolation and cold maceration process based on the nature of phytoconstituents present in herbs were adopted in the present work. A combination of extracts may produce a synergistic effect in the treatment of disease. The valuable medicinal properties of secondary metabolites were accumulated in medicinal plants and utilized to produce specific action against the treatment of diseases.

The present work was designed to formulate extract composition for reduction of oxidative stress, which is the main risk factor in the development of hypertension or high blood pressure. Persistent hypertension is a major cause of chronic kidney disease and a risk factor for strokes, heart failure, and arterial aneurysm. A moderate increase in arterial blood pressure reduces the average lifespan. Dietary and lifestyle changes and medications can help control blood pressure and reduce the risk of health problems.[2] Drugs with anti-oxidant/free radical scavenging activity show benefit in reduction of harmful effects of stress.

Polyherboceutical formulation, composed of six different plant extracts mentioned in ayurvedic textbooks for blood pressure management properties, were used in this study. Simultaneously pharmaco-analytical tests and anti-oxidant profiles were determined.

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Materials and Methods

Plant Material

The raw plant materials were purchased from a local market in Jaipur (Rajasthan). These were authenticated by a qualified botanist from Department of Botany, University of Rajasthan, Jaipur. The voucher specimen and identification number are given in Table 1.

Capsule Shells

Hard Gelatin capsule shells (Pharma grade) were also purchased from a vendor in Jaipur. The capsule shells were de-dusted and transferred into polybags, labeled, and stored in a controlled environment (25°C, relative humidity not more than 50%).

Chemical and Reagents

All the chemical reagents and standards were procured from Sigma Aldrich, Merck, JT baker, and TCI to maintain a high level of accuracy and precision during analysis.

Size Reduction and Extraction Process

Each raw material was ground in the Pulveriser (50 kg/hr.) to a coarse powder (14 Mesh). The extraction method was selected based on the nature of raw materials. Four raw materials, namely *Rauwolfia serpentina*, *Terminalia arjuna*, *Boerhaavia diffusa,* and *Convolvulus pluricaulis* have non-volatile active constituents; therefore, hot decoction method was adopted.

Remaining two raw materials were *Nardostachys jatamansi,* and *Centella asiatica* have volatile active constituents; therefore, the cold maceration method was adopted. The composition of extracts is shown in Table 2.

Hot Decoction Method[3]

Coarse powder of each of *Rauwolfia serpentina*, *Terminalia arjuna*, *Boerhaaviadiffusa,* and *Convolvulus pluricaulis*) was mixed. 20 kg of material was loaded in a vertical extractor (100 L) unit and extracted (soaked) in 60 L of demineralized water (1:3 ratio). The mixture was heated at 70–75°C for 3 hours and then filtered by passing it through a nylon cloth (200 mesh). This process was performed in triplicate, and all filtrates were mixed in a separate vertical distillation unit.

Combined liquid extract was concentrated at reduced temperature (60–65°C) and pressure (10 mbar) to obtain a thick paste. The paste was dried in a vacuum tray drier at 70°C temperature for 05 hours to obtain dried lumps. The lumps were ground in a pulveriser to obtained a fine powder of extract.

Cold Maceration Method[3]

Coarse powder of (*N. jatamansi* and *C. asiatica*) was mixed with 4 times (1:4 ratio) of demineralized water in a 50 L percolator and kept overnight for maceration at room temperature. The liquid extract was filtered by passing it through a 200 mesh nylon cloth to obtain a clear liquid extract.

Trituration and Encapsulation[3]

The liquid filtrate obtained in cold maceration was mixed with solid powdered extract in a ratio of 4:1, obtained from hot percolation in an edge runner machine. Trituration was carried out to encapsulate (or coat) the active constituents of the liquid extract on solid powder extract. The process is mentioned as "*Bhavna"* in Ayurveda texts. Uniform thick paste of the pooled extract was obtained after 3 hours of trituration. Thick paste was dried in hot air oven at 50°C

Table 1: Details of used plant materials with authentication

S. No.	*Common name*	*Botanical name*	*Family*	*Useful part of plant*	*Identification number*
1	Sarpagandha	*Rauwolfia serpentina*	Apocynaceae	Root	RUBL211628
2	Arjuna	*Terminalia arjuna*	Combretaceae	Bark	RUBL211626
3	Purnarnava	*Boerhaaviadiffusa*	Nyctaginaceae	Root	RUBL211627
4	Shankhpushpi	*Convolvulus pluricaulis*	Convolvulaceae	Whole plant	RUBL211629
5	Mandukparni	*Centella asiatica*	Apiaceae	Whole plant	RUBL211625
6	Jatamansi	*Nardostachysjatamansi*	Valerianaceae	Rhizome	RUBL211624

Table 2: Composition of ingredients in polyherboceutical formulation

S. No.	*Name of ingredients*	*Quantity of raw material (kg)*	*Type of extraction*	*Extraction ratio*	*Quantity of ingredients*
1	*Rauwolfia serpentina*	10.0	Hot percolation	1:10	200 mg
2	*Terminalia arjuna*	5.0	Hot percolation	1:8.5	100 mg
3	*Boerhaaviadiffusa*	2.5	Hot percolation	1:10.5	50 mg
4	*Nardostacysjatamansi*	2.5	Cold maceration	1:7.5	50 mg
5	*Centella asiatica*	2.5	Cold maceration	1:16	50 mg
6	*Convovuluspluricaulis*	2.5	Hot percolation	1:14	50 mg
	Total	25.0			500 mg

for 12 hours. The dried lumps thus obtained were ground in a grinder and shifted (sieve mesh size 40#) to obtain fine powder.

Capsule Filling and Packing

The capsules were filled in a manual capsule filling machine (300 capsules in a single operation) under a controlled environment (25 ± 5°C and less than 60% relative humidity). The filled capsules were de-dusted, sealed, and stored in bottles and containing silica gel packets for moisture-free storage.

Physio-chemical Analysis[4]

The physio-chemical investigations of the polyherboceutical formulation were carried out as determination of extractive values, ash values, loss on drying, weight variation, pH, disintegration time and total fat content.

Total Tannin Content[5]

The Folin-ciocalteu method was used to determine the total tannin content. Tannic acid solutions (20 to 100 µg/mL) were prepared as a standard set. Using a UV-visible spectrophotometer, the absorbance of standard and test solutions was measured with a blank at 725 nm wavelength. The total tannin content of the polyherboceutical formulation was calculated as mg of tannic acid equivalent (TAE)/g.

Total Bitter Content[6]

A gravimetric process was followed to determine the total bitter content.

Total Alkaloid Content[7]

A gravimetric extraction process followed to determine the total alkaloid content.

Total Flavonoid Content or Quercetin Equivalent[8]

The total flavonoid content was determined by using an aluminum chloride complex-forming assay. Polyherboceutical formulation and standard quercetin were dissolved in methanol and processed to calculate as quercetin equivalents (%QE). A total of 10% solution of aluminum chloride was added to each prepared dilution and allowed to stand for 5 minutes after which 200 µL solution of 1M sodium hydroxide was added consecutively. A UV-visible spectrophotometer was used to calculate the absorbance of this reaction mixture at 510 nm. The total flavonoid content in polyherboceutical formulations was estimated using a standard quercetin curve and regression equation.

Total Phenolic Content or Gallic Acid Equivalent[9]

The total phenolic content of polyherboceutical formulation was determined by using Folin-Ciocalteu method. The absorbance was measured at 765 nm using UV-visible spectrophotometer. The standard gallic acid curve and regression equation were used to calculate total polyphenol content in polyherboceutical formulation.

Microbial Contamination[4]

Total bacterial count, fungal count, and pathogen analysis were performed to ensure the viable count of microorganisms and limits followed as prescribed in Ayurvedic Pharmacopoeia of India.

Fourier Transform Infrared Spectroscopy Study[10]

Fourier transform infrared spectrophotometer (FTIR) is an effective technique for identifying the types of chemical bonds (functional groups) existing in metabolites. Sample powder of polyherboceutical formulation was analyzed using FTIR fingerprinting. Spectra generated by using Thermo Scientific, Nicolet iS 10 FTIR spectrometer in which 5 mg of powdered mass were placed over the ZnS crystal of iTR basic technique also defined as Attenuated total reflectance (ATR) technique and scanned over the range of 4000 to 400 cm^{-1} by which IR spectra obtained in Thermo Insight software.

In vitro Anti-oxidant Activity (DPPH Radical Scavenging Activity)[9]

Free radical scavenging activity of polyherboceutical formulation was performed as per the method of "Blois" with a few alterations. In brief, a 0.2 mM DPPH radical solution in methanol was prepared, and then 3 mL of this solution was mixed with 3 mL of the sample solution in altered concentrations (1–15 mg/mL) of the formulation. This mixture was kept in the dark for 30 minutes; the absorbance was measured at 517 nm. The DPPH solution absorbance is inversely proportional to the DPPH radical scavenging activity. The DPPH solution without sample solution was used as a control. Gallic acid was used as a standard in the concentration of 0.005–0.5 mg/mL. Inhibition concentration at 50% (IC_{50}) was calculated by plotting a calibration curve with concentration and percentage inhibition at each point. Percentage inhibition was calculated for standard and polyherboceutical formulation by using the given formula.

% inhibition = [(Absorbance of blank – Absorbance of formulation) ×100] / Absorbance of blank

In vitro Anti-oxidant Activity (Reducing Power Assay)[11]

The reducing power of polyherboceutical formulation was determined by the method of "Oyaizu" with some modifications. The reduction of ferric to ferrous ion is directly proportional to the absorbance indicating the sample (3–20 mg/mL) in double-distilled water was mixed 2.5 mL of phosphate buffer incubated at 50°C for 20 minutes after which 1.5 mL of trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes from all the tubes, 0.5 mL of ferric chloride was added. The absorbance was measured at 700 nm on a spectrophotometer. Gallic acid was used as a standard for comparison at a concentration of 0.12–0.36 mg/mL. The water was used as a blank instead

of additives. Comparative evaluation of concentration value calculated at 0.5 of absorbance for standard and polyherboceutical formulation.

Result and Discussion

In-process Quality Control

In the process, quality control tests were performed to ascertain the performance of the formulation. Observations are shown in Table 3.

Quality Control Parameters

The details of quality control tests were performed on the three batches of polyherboceutical formulations shown in Table 4. The quality control parameters included physicochemical parameters, qualitative, and quantitative analysis, microbiological parameters, and comparative results for the evaluation of uniformity and batch to batch consistency.

Total Flavonoid Content

The absorbance on concentration curve for to linear with the quercetin standard. The concentration is expressed as mg/mL on X-axis and the corresponding observed absorbance on Y-axis. The flavonoid concentration of sample determined by extrapolation was calculated as 1.83% w/w given in Table 4 and quercetine linear curve are shown in Fig. 1.

Table 3: Analytical data for in-process quality control

			Sampling time		
Sr. No.	*Test*	*Acceptance criteria*	*Initial*	*Middle of batch*	*Completion of batch*
1.	Description	A red Coloured hard gelatin capsule of '0' size, Brown color Powder	Complies	Complies	Complies
2.	Uniformity of weight	Average weight ± 10 % w/w	Complies	Complies	Complies
3.	Disintegration time	NMT 30 minutes	8–9 minutes	7–8 minutes	7–8 minutes

Table 4: Analytical Result of Polyherboceutical Formulation:

Description			
Appearance	Hard gelatin capsule		
Color	Red Body & Cap containing blackish-brown powder		
Odor	Characteristic		
Taste	Bitter		
Test parameters	*Batch no.1*	*Batch no.2*	*Batch no.3*
pH value (1% weight/volume in water)	4.71	4.73	4.71
Loss on drying	6.01%w/w	5.71%w/w	7.65%w/w
Total ash	12.96 % w/w	12.60 % w/w	12.78 % w/w
Acid insoluble ash	3.44% w/w	3.66% w/w	3.32% w/w
Water soluble ash	4.89%w/w	4.19%w/w	4.45%w/w
Water soluble extractive	37.78%w/w	37.91%w/w	37.55%w/w
Alcohol soluble extractive	24.18%w/w	24.17%w/w	24.15%w/w
Weight variation	-3.15% to +4.40%	-3.30% to +4.51%	-4.02% to +3.85%
Disintegration time	7–8 min	7–8 min	7–8 min
Total alkaloids content	0.16% w/w	0.17% w/w	0.17% w/w
Total tannin content	0.08 % w/w	0.07 % w/w	0.09 % w/w
Total bitter content	6.27%w/w	6.24%w/w	6.26%w/w
Total flavonoids content	1.83 % w/w	1.82%w/w	1.83% w/w
Total polyphenol content	0.11 % w/w	0.10%w/w	0.12% w/w
Total bacterial count	2500 cfu/g	2200 cfu/g	2400 cfu/g
Total fungal count	70 cfu/g	60 cfu/g	60 cfu/g
E. coli	Absent/g	Absent/g	Absent/g
Salmonella	Absent/g	Absent/g	Absent/g
S. aureus	Absent/g	Absent/g	Absent/g
Ps. aeruginosa	Absent/g	Absent/g	Absent/g

Total Polyphenols Content

The absorbance on concentration curve for to linear with the gallic acid standard. The concentration is expressed as mg/mL on X-axis, and the corresponding observed absorbance on Y-axis. The polyphenol concentration of the sample determined by extrapolation was calculated as 0.11% w/w given in Table 4, and gallic acid linear curve is shown in Fig. 2.

Anti-oxidant Activity

Anti-oxidant activities were determined using two different methods: DPPH free radical scavenging activity and ferric ion reducing power assay (FRAC) with gallic acid serving as a standard natural anti-oxidant compound. The anti-oxidant power of the sample was evaluated with a comparison of inhibition concentration at 50% and 0.5 of absorbance value with standard gallic acid (Fig. 3). Polyherboceutical formulation had an IC_{50} value of 7.87 mg/mL in scavenges DPPH free radical (Fig. 4). Ferric ion reduction property was also much effective and found 0.5 of absorbance at 8.6 mg/mL. Good anti-oxidant property may be high amounts of total flavonoids, polyphenols, and tannins present in a sample. The results are given in Tables 5-7. It is evident that reduction of oxidative stress is directly correlated with anti-hypertensive activities of Polyherboceutical formulation[12]

Table 5: DPPH free radical scavenging activity of standard gallic acid and polyherboceutical formulation

Name of sample	*Concentration (mg/mL)*	*% Inhibition*	*IC_{50}*
Gallic acid	Control	-	mg/ml
	0.0005	10.54	
	0.001	21.43	
	0.002	54.26	0.00195
	0.003	88.43	
	0.004	93.74	
Methanolic extract of formulation	1	23.48	
	5	44.11	7.87
	10	56.53	
	15	74.08	

Table 6: Reducing power assay of gallic acid and methanolic extract of polyherboceutical formulation

Name of sample	*Concentration (mg/mL)*	*Absorbance*	*0.5 of absorbance*
Gallic acid	0.012	0.184	0.036
	0.024	0.674	
	0.036	0.937	
Methanolic extract of formulation	3.26	0.249	8.643
	8.15	0.526	
	16.31	0.798	

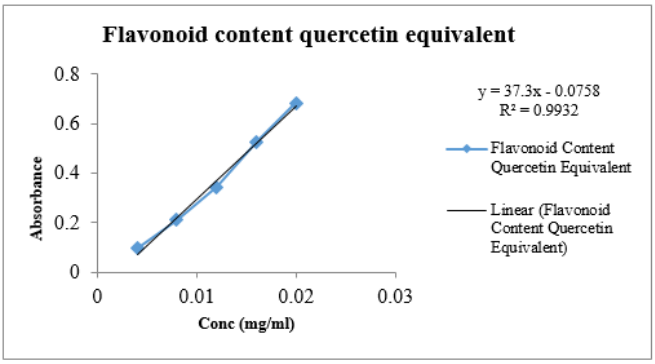

Fig. 1: Standard quercetin curve

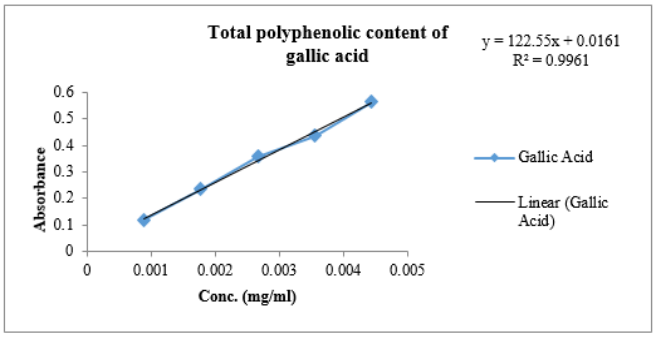

Fig. 2: Standard gallic acid curve

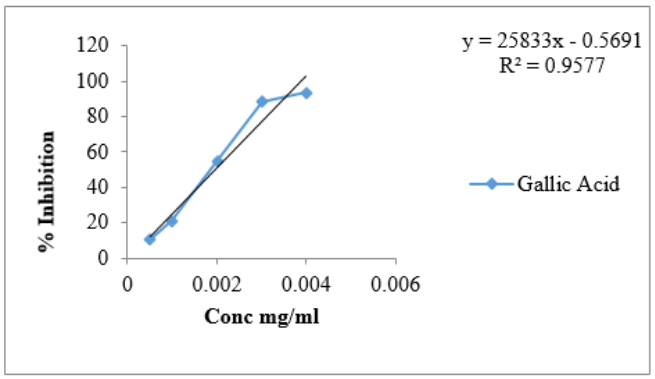

Fig. 3: Radical scavenging activity of gallic acid, X axis shows c oncentration in mg/mL and Y axis shows % inhibition obtained for each sample in the formulation is presented.

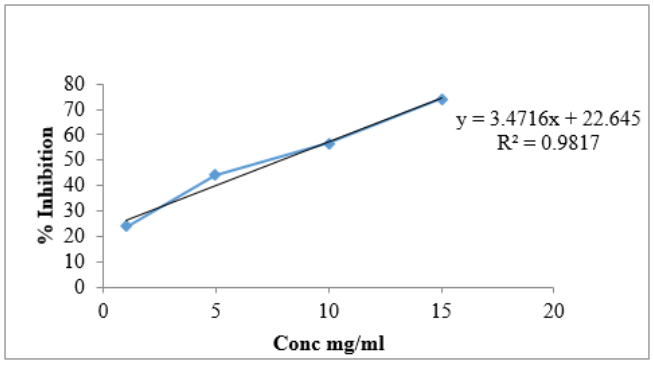

Fig. 4: Radical scavenging activity of methanolic extract of Polyherboceutical formulation, X axis shows the concentration in mg/mL and Y-axis shows % inhibition obtained for each sample in the formulation is presented.

Table 7: FTIR observations

S. No.	Absorption valleys in wave numbers	Possible functional group	Possible lead components
1.	3314.36	N-H Stretch	Sec. amine
2.	2980.94	N-H Stretch	Amine salt
		C-H Stretch	Alkane
3.	2866.26	C-H Stretch	Alkane
4.	2825.22	C-H Stretch	Alkane
5.	1600.38	C-H Stretch	Aldehyde
		0-H Bend	Phenol
6.	1318.80	S=O Stretch	Sulfone
		C-N Stretch	Aromatic amine
		C-F Stretch	Fluro compound
7.	1261.08	C-O Stretch	Aromatic ester
		C-O Stretch	Alkyl aryl ester
8.	804.38	C=C Bend	Alkene (Trisubstituted)
9.	704.48	C-H Bend	Mono substituted benzene derivative
10.	599.51		
11.	599.07		
12.	463.16	C-C stretch	Aliphatic carbon chain
13.	445.81		
14.	429.06		
15.	417.79		

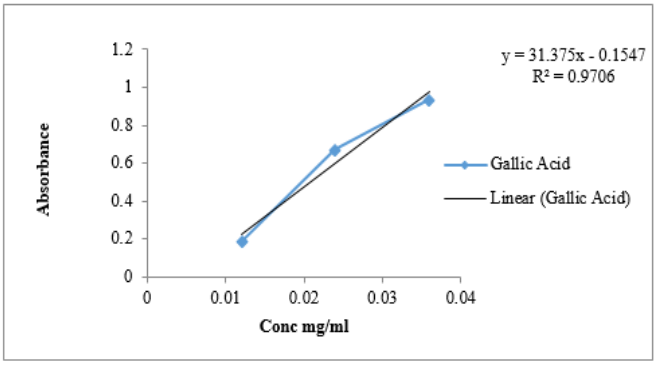

Fig. 5: Ferric reducing the anti-oxidant capacity of gallic acid.

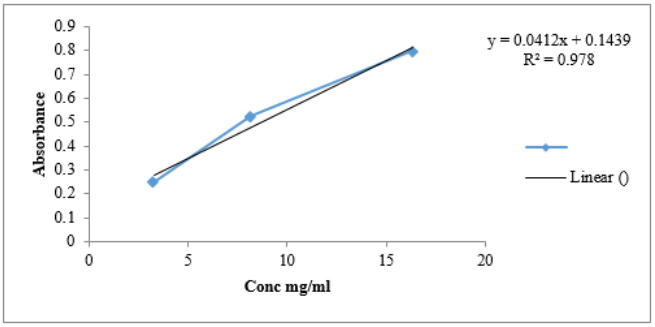

Fig. 6: Ferric reducing the anti-oxidant capacity of methanolic extract of Polyherboceutical formulation

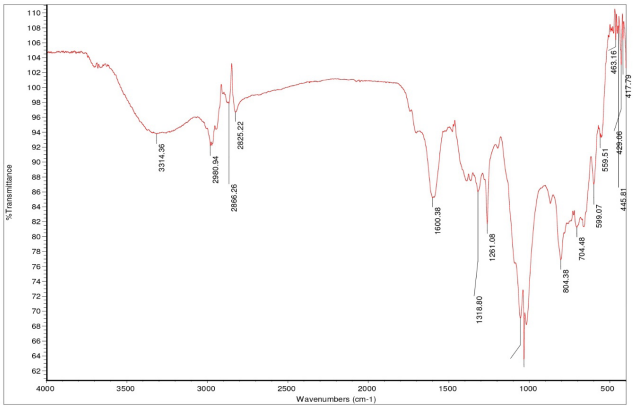

Fig. 7: FTIR spectrum of polyherboceutical formulation.

FTIR Scanning

The FTIR fingerprint profile of polyherboceutical formulation was identified majorly. It characterized as nitrogenous, aromatic, and unsaturated components, which might be helpful for structural elucidation of active phytochemicals present in extracts of herbs. Spectrum identified with major valleys on given wave numbers shown in Fig. 7 and their corresponding values with functional groups given in Table 7.

Conclusion

The present work was undertaken to establish the various phytochemical, physio-chemical, FTIR spectrum and spectrum activity, which could further be for both research institutes and pharmaceutical industries in the manufacturing-specific and targeted bioactive molecules from polyherboceutical formulation. In the present study, polyherboceutical formulation was evaluated for its ability to reduce oxidative stress. Oxidative stress is an important indicator for the pathogenesis of hypertension, and by reducing it, anti-oxidants may have been shown to be beneficial in the management of hypertension.[12]

Therefore, it can be concluded that the developed polyherboceutical formulation, including six above potent ingredients, possesses useful characteristics in capsule formulation with good anti-oxidant activity, and it is quite likely that it may also have anti-hypertensive potential.

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Author Contributions

Patent has been granted by Patent Office, Intellectual Property Rights, Government of India related the present work done to the author, Mr. Naveen Kumar Garg as

Inventor and all work has also been published in the Journal of Patent office. (Indian Patent number: 312803, dated 16.03.2019)

References

1. Rajpal V. Standardization of Botanicals, vol. 1, 1st edition, New Delhi, Business horizons pharmaceutical publishers, 2011; 212-218.
2. Tabassum N, Ahmad F. Role of natural herbs in the treatment of hypertension. Pharmacogn. Rev. 2011; 5(9): 30-40.
3. Garg NK, Gupta S, Khandelwal NK. Future magic bullet of "Polyherboceutical Formulation" for treatment of hypertension. The patent office Journal. 2012; 39: 16094.
4. The Laboratory guide for analysis of Ayurveda, Unani & Siddha formulations, Central council for Research in Ayurveda and Siddha, New Delhi, published by Ministry of AYUSH. 2010: 28-64.
5. Selvakumar S, Vimalanban S, Balakrishnan G. Quantitative determination of phytochemical constituents from *Anisomeles malabarica*. MOJ Bioequivalence & Bioavailability. 2019; 6(1): 19-21.
6. Rane R, Gangolli D, Salkar K, Shelar R, Kundalwal S, Chotalia C, Salvi R. Analytical evaluation of *Tinospora cordifolia* extract and capsule, I**nt**. J. Herb. Med. 2017; 5(3): 53-57.
7. Reeta K, Brijesh R, Anita R, Sonal B. *Rauvolfia serpentina* L. Benth. ex Kurz.; Phytochemical, pharmacological and therapeutic aspects. Int. J. Pharm. Sci. Rev. Res. 2013; 23(2) 56: 348-355.
8. Ovais US, Muhammad M, Ali KK, Nor A, Mohd S, Mohd Nur NA. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. J. Pharmacogn. Phytochem. 2014; 3 (3): 104-108.
9. Suman C, Shabana K, Bharathi A, Hemant L, Min HY, Mahmoud A, El Sohly, Ikhlas AK, Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. Evid.-Based Complementary Altern. Med. 2014; Article ID 253875: 9 pages.
10. Pravin KP, Sadasivan P, Mathews MK, Pawan K, Shabeer SI, Anand T, Cherian KM. Fourier transform infra-red (FT-IR) spectral studies of novel poly-herbal formulation of anti-obesity drug. JMST. 2015; 4(1): 55-57.
11. Irshad M, Zafaryab M, Singh M, Rizvi M. Comparative analysis of the antioxidant activity of Cassia fistula extracts. International journal of medicinal chemistry. 2012;2012.
12. Baradaran A, Nasri H, Rafieian-Kopaei M. Oxidative stress and hypertension: possibility of hypertension therapy with antioxidants, J. Res. Med. Sci. 2014; 19(4): 358–367.

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