Original Research Paper

Volume - 12 | Issue - 02 | February - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Ayurveda

PHYTOCHEMICAL SCREENING, FORMULATION AND EVALUATION OF HERBAL DOSAGE FORM CONTAINING ALOE VERA LEAF EXTRACT

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ABSTRACT Medicinal plants have curative properties due to the presence of the various complex chemical substances of different compositions, which are found as secondary plant metabolites in one or more parts of these plants. *Aloe vera* Linn., Liliaceae is an annual extensive perennial climber with large ovoid and tuberous leaf herb indigenous to India and widely used in the treatments of free radical generation, for debility. It was also found to have alterative, aphrodisiac, cholagogue, demulcent, diuretic, rejuvenating actions. The present paper deals with the formulation and evaluation of the antioxidant activity of tablets prepared from aqueous extract of the selected plant. A solid pharmaceutical dosage formulation using a novel dry plant extract (tuberous leaves) using various excipients viz., carbopol, ethylcellulose, MCC, dibasic calcium phosphate, and PEG-4000 by direct compression was reported to be statically significant as antioxidant activity. The present communication also deals with the evaluation of formulated tablets (weight variation, friability, hardness, and disintegration time).

KEYWORDS : Oxidants, *Aloe vera*, tablets, antioxidant activity, PEG, MCC, Carbopol, Herbal formulation.

INTRODUCTION

Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history [1-4]. It was an integral part of the development of modern civilization. A primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopeias [5]. Indeed, well into the 20th century much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances. WHO report 80% of the world population relies on the drug from natural origin. Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer, and AIDS [6, 7]. Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routes for producing free radicals in food, drugs, and still living systems. Catalase and hydroperoxides enzymes change hydrogen peroxide and hydroperoxides to nonradical forms and purpose as natural antioxidants in the human body. Owing to the depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential [8-11]. At present available synthetic antioxidants similar to butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinone, and gallic acid esters have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity [12,13]. Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical-induced tissue injury. Polyphenolic compounds with known properties include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action [14]. Several confirmations suggest that the biological actions of these compounds are related to their antioxidant activity [15]. An easy, rapid, and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydroxyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron, and the absorbance decreases [16]. In particular, despite the extensive use of wild plants as medicines in Iran, the prose contains few reports of antioxidant activity and chemical composition of these plants. In the current study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plant species, which are being used traditionally.

MATERIALS AND METHODS

The tuberous leaves of the plant *Aloe vera* Linn. were collected from the Rai Bareilly district UP, in September 2009. The plant was then authenticated by the taxonomist of the department, India. Ethylcellulose, Carbopol, Microcrystalline Cellulose are procured by Colorcon Asia Pvt. Ltd - Mumbai - India, Polyethylene Glycol Dibasic Calcium Phosphate, Methylparaben are procured by micro labs, Hosur, T. N.

Extraction Process

The preliminary phytochemical screening of the plant involves extraction of the plant material and identification of the plant's active constituents. Continuous hot percolation process by using Soxhlet apparatus.

Petroleum Ether Extract

The shade-dried coarsely powdered plants tuberous leave of *Aloe vera* Linn. (120 gms) were extracted with petroleum ether (32°C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The yellowish brown-colored residue was obtained. The residue was then stored in a desiccators.

Acetone Extract

The shade-dried coarsely powdered plants' tuberous leaves of *Aloe vera* Linn. (120 gms) were extracted with acetone (55-56°C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The yellowish brown-colored residue was obtained. The residue was then stored in a desiccators.

Alcohol Extract

The shade-dried coarsely powdered tuberous leaves of *Aloe vera Linn.* (120 gms) were extracted with alcohol (75-78°C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The yellowish brown-colored residue was obtained. The residue was then stored in a desiccators.

Aqueous Extract

The marc left after Petroleum ether extraction of tuberous leaves of *Aloe vera* Linn. was dried and extracted with Chloroform water (2.5 ml in one-liter I.P) by cold maceration process in a narrow mouth bottle for 3 days. After completion of the extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. The dull yellow-colored residue was obtained and it was stored in a desiccator.

Test for carbohydrates

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

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 the presence of carbohydrates.

Barfoed's test: The Barford's solution added to 0.5 ml of the solution under examination, heated to a boil. The formation of a red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of the drug. A green or blue color indicated the presence of carbohydrates.

Test for alkaloids

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.

Wagner's test: Acidify the extract of the drug with 1.5 % v/v of hydrochloric acid and added a few drops of Wagner's reagent (iodine potassium iodide solution). Formations of reddish-brown precipitate indicated the presence of alkaloids.

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.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. The pink-red color indicated the presence of glycosides.

Baljet's test:

To the drug extract, sodium picrate solution was added, yellow to orange color was indicated the presence of glycosides.

Borntrager's test: Few ml of the dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then the organic layer was separated to which ammonia was added, pink, red, or violet color was produced in the orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing a trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. The blue color in the acetic acid layer and red color at the junction of the two liquids indicated the presence of glycosides.

Test of saponins

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

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of oil. hydrochloric acid followed by a small piece of magnesium. In presence of flavonoids a pink, reddish-pink, or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish-black color indicated the presence of tannins.

Test for protein and amino acid

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 sulfate solution mix thoroughly, a purplish - violet or pinkish-violet color produced that indicates the presence of proteins.

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

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 cools the solution carefully. Added 20 % of sodium hydroxide solution in excess orange color indicated the presence of aromatic amino acid.

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

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled then added 1 ml of lead acetate solution, the gray-black precipitate was formed which indicated the presence of sulfur-containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulfate solution then added 10 % sodium hydroxide solution a clear blue solution was obtained which showed glycerin present in the sample.

Using sodium hydrogen sulfate: The extract was taken in the test tube added a pinch of sodium hydrogen sulfate pungent odor was formed which showed glycerin present in the sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shake vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Formulation of Tablets

The plant extract was mixed with the excipients and compressed into tablets. The details of the composition were given in table 1.

Table 1: Formulation of Tablets

Ingredients	BATCH NO.					
	QUANTITY PER TABLET (mg)					
	F1	F2	F3	F4	F5	F6
Plant extract	300	300	300	300	300	300
Carbopol	40	40	40	-	-	-
Ethyl cellulose	-	-	-	40	40	40
Microcrystalline cellulose	70	70	70	70	70	70
Dibasic calcium phosphate	70	70	70	70	70	70
Peg 4000	20	20	20	20	20	20
Methyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Weight per tablet	**500**	**500**	**500**	**500**	**500**	**500**

***In-vitro* antioxidant activity of both extracts**

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydroxyl radical (DPPH) was used for the determination of the free radical-scavenging activity of the extracts [17]. Different concentrations of each herbal extract were added, at an equal volume, to the methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. BHT and quercetin were used as standard controls. IC_{50} values denote the concentration of the sample, which is required to scavenge 50 % of DPPH free radicals.

Statistical Evaluation

The data were statically analyzed by one-way ANNOVA followed by

Dunnet's t-test and values were considered significant. And values were expressed ± SEM. And $p<0.05$

RESULTS AND DISCUSSION

Antioxidant plants an important role in inhibiting glucose levels and inflammation thus protecting humans against free radicals generation. Realizing the fact this research was carried out to evaluate the antioxidant activity of aqueous extract tablets of tuberous leaves of *Aloe vera* Linn. in the DPPH free radical scavenging model. The Phytoconstituents were extracted by using a different solvent of increasing polarity like petroleum ether, acetone, alcohol, and water were presented.

Phytochemical Investigation

Table 3: Percentage yield of extraction of tuberous leafs of *Aloe vera* Linn.

Plant used	Part used	Percentage yield			
		Pet. ether	acetone	Alcohol	Distilled water
Aloe vera Linn.	leafs	2.18	3.7	3.1	17.10

Table 4: Preliminary Phytochemical screening of both extracts of tuberous leafs of *Aloe vera* Linn.

Name of extract	Pet. Ether	Acetone	Alcohol	Distilled water
Carbohydrate	-	-	+	+
Glycosides	-	-	+	+
Fixed oils & Fats	-	-	-	-
Proteins & Amino acids	-	-	-	+
Saponins	-	-	-	+
Resins	-	-	+	-
Phytosterol	-	-	+	+
Alkaloids	-	+	+	+
Flavonoids	-	-	+	+
Gum & mucilages	-	-	+	+

+ = Presence ; - = Absence

The phytochemical evaluation shows the presence of Fixed oil, Carbohydrate, Tannins, Phenolic compound, Alkaloids, Saponins, Sterols and Flavonoids in acetone alcohol and aqueous extract. But most of these compounds were found in the aqueous extract. Hence, only the aqueous extract was selected for the formulation of the tablets [18].

Table 5: Evaluation of Powder Blend

Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F_1	0.42	0.53	8.10	1.01	31.2
F_2	0.46	0.51	16.52	1.03	33.0
F_3	0.41	0.54	17.08	1.59	33.79
F_4	0.49	0.59	18.24	1.49	35.90
F_5	0.50	0.53	14.47	1.23	28.57
F_6	0.43	0.60	12.46	1.69	30.36

Table 6: Evaluation of Tablets

Batch	Hardness (kg/cm^2)	Thickness (mm^2)	% Weight Variation	% Friability	Disintegration Time
F_1	4.04	3.6	2.49	0.80	9 min 55 sec
F_2	4.05	3.9	2.30	0.71	12 min 17 sec
F_3	4.19	3.3	1.90	0.86	8 min 13 sec
F_4	4.12	3.6	1.50	0.68	11min 28 sec
F_5	4.03	3.8	2.41	0.89	13 min 31 sec
F_6	4.10	3.3	2.21	0.88	10 min 38 sec

Preformulation studies Evaluation of powder blend

The granules obtained for the trial batches (F1-F6) were satisfactory. No rat holing was observed during the flow of granules from the hopper. Capping and sticking were not observed. From the compressibility index and Hausner's ratio values obtained for granules of batches F1– F6, the granules were found to have good flow properties.

Evaluation of Tablets

The tablets were compressed at the specified weight (500 mg). The maximum weight variation of the tablets was ± 2.71%, which falls within the acceptable weight variation range of ± 5%, hence the tablets of all batches passed the weight variation test. Hardness for tablets of all batches was in the range of 4.0 to 4.2 kg/cm^2, which falls above the limit of not less than 3.0 kg/cm^2. The friability value for tablets of none of the batches was more than 0.8 7%. The thickness of the tablets of all the batches was found in the range of 3.4 - 3.7 mm indicating fairly acceptable tablets. Disintegration time is an important parameter of the tablet. An ideal tablet should disintegrate within 15 min. The tablets of all the batches disintegrated within 13 minutes 30 seconds.

Antioxidant activity

Table 7: Comparison of DPPH radical scavenging activity of the plant extracts and those of BHT and quercetin

Plant species	Concentration (mg/ml)	Scavenging (%)
Leaf extract	0.15	94.3±0.1[1]
Formulated tablet	0.79	91.5±0.3
BHT	0.41	92±0.7
Quercetin	0.039	94.6±0.8

1Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

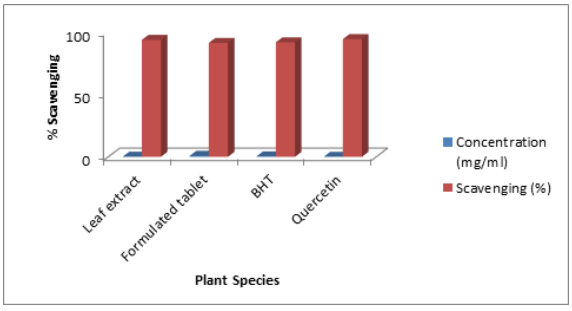

Fig. 1: Comparison of DPPH radical scavenging activity of the plant extracts and those of BHT and quercetin

Free radicals are concerned with many disorders like neurodegen erative diseases, cancer, and AIDS. Antioxidants during their scavenging power are helpful for the management of those diseases. DPPH stable free radical method is an easy, rapid and receptive way to survey the antioxidant activity of a precise compound of plant extracts [18-19]. IC_{50} of the standard compounds, BHT and quercetin were 0.044 and 0.01 mg ml-1, respectively. The uppermost radical scavenging activity was shown by leaf extract with IC_{50}=0.018 mg ml^{-1} which is higher than that of BHT ($P<0.05$). The radical scavenging activity in the plant extracts decreased in the subsequent order: *Aloe vera extract* > Formulated tablet. Most of the plant's extracts at dissimilar concentrations exhibited more than 75 % scavenging activity (Table 7). The radical scavenging effect of *Aloe vera* leaf extract at 0.1 mg ml^{-1} was similar to BHT at 0.4 mg ml^{-1}. Therefore, the antioxidant effect of *Aloe vera* leaf extract was 4 times greater than that of the synthetic antioxidant, BHT.

CONCLUSION

The result of the current study showed that the extract of *Aloe vera* leaf extract, which holds the highest amount of flavonoid and phenolic compounds, exhibited the maximum antioxidant activity. The high scavenging property of *Aloe vera* leaf extract may be due to hydroxyl groups accessible in the phenolic compounds' chemical structure that can offer the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a probable anticipatory intervention for the diseases. All of the extracts in this research exhibited a dissimilar amount of antioxidant activity. *Aloe vera* leaf extract showed a higher potency than BHT in scavenging of DPPH free radicals. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

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