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Development and Evaluation of Antioxidant Activity Polyherbal Formulation

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ABSTRACT:
The goal of this study was to create a polyherbal formulation (PHF) with four different herbs and assess its phytochemicals, physical constants, and antioxidant activity using the DPPH method.The morphological and pharmacognostic characteristics of the PHF authenticated herbs were studied. The combination extract contained alkaloids, glycosides, carbohydrates, amino acids, tannin, steroids, and flavonoids, according to phytochemical screening. Loss on drying (LOD), pH, ash values, LOD, and extractive value have all been investigated. Using the DPPH free radical scavenging technique, the antioxidant activity of the combined extracts (100 mg each) was evaluated.When compared to ascorbic acid as the reference standard, the results showed that the combination extract has the best antioxidant action at a dose of 400 g/ml. When compared to ascorbic acid as the reference standard, the results of this study clearly revealed that the combination extract had the best antioxidant action at a dose of 400 g/ml.

Keywords: Polyherbal formulation (PHF), Phytochemical, Antioxidant activity, Ascorbic acid.

I. INTRODUCTION

There has been a lot of progress in the field of herbal medicine in the last few years. Because of its natural origins and fewer side effects, it is gaining popularity in developing countries [1]. It is classified in the old Indian medical system. A substance capable of preventing the oxidation of other molecules is known as an antioxidant. Oxidation is a chemical reaction in which electrons are transferred from a material to an oxidizer. Free radicals can be produced during oxidation reactions. As a result, these radicals can trigger chain reactions that harm cells. Antioxidants stop these chain events from continuing by eliminating free radicals and inhibiting other oxidation reactions. Antioxidants, such as thiols, ascorbic acid, and polyphenols, work as reducing agents by getting oxidised themselves. The antioxidant activity of plants has been linked to their phenolic content in numerous studies. Plants included phenolic chemicals, which can act as antioxidants [2]. The oxidative process is the most prevalent way for food, medications, and even living systems to produce free radicals [3]. Oxygen radicals make up the majority of free radicals that harm biological systems. Radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, and metal chelating agents are all functions of antioxidants [4]. Natural antioxidants (safe and nontoxic) are preferred over synthetic antioxidants because of their effect on the immune system (toxic for human). Plants have a variety of elements that have a local physical effect on bodily tissues, and the topical application of herbal treatments is one of the most visible in the simplest health-care practises [5]. The antioxidant activity of Emblica officinalis fruits, Eugenia jambolana. bark, Terminalia chebula fruits, and Acacia catechu bark of Indian origin was investigated to support the use of selected plant extracts in Ayurveda. The goal of this study was to determine the antioxidant activity of a blend of extracts (polyherbal formulation [PHF]) in vitro and compare it to that of ascorbic acid, a well-known antioxidant.

II. METHODS

Plant material and authentication

The plant material was obtained from the Buldana local market. The plants were authenticated by Dr. M.R. Bhise with reference letter no. DOB/2018-19/01 from the Botanic Department, L. K. D. K Banmeru Science College, Lonar, Dist-Buldana (MS). The plant parts have been separated, completely washed with tap water, shade dried and homogenised to fine powder for further studies and stored in a sealed container.

Extraction of Plant Material

Plant components from Emblica officinalis fruits, Eugenia jambolana. bark, Terminalia chebula fruits, and Acacia catechu bark

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were cut into slices, dried in the shade, ground into a coarse powder, and passed through an 80 mesh filter. The powdered plant materials were extracted with various solvents using various extraction processes such as the soxhlet device, maceration, and percolation, and then subjected to phytochemical screening and further analysis.

Preliminary Phytochemical Screening[5]

Carbohydrate, amino acids, cardiac glycosides, alkaloids, saponins, phenols, flavonoids, steroids, terpenoids, tannins, anthraquinones, quinones, lipids, and volatile oils were detected in the extracts using qualitative phytochemical analysis. To discover the phytochemicals included in the herbal preparation, preliminary phytochemical investigations were conducted using established techniques. The polyherbal formulation was discovered to contain essential phytochemicals such as alkaloids, flavanoids, phenols, and other anti-oxidant compounds.

Physical evaluation of extract

The physical evaluation of the combination of all extract was done for following parameters. The results are shown in Table 1.

1. Moisture content (Loss on drying at 105°C).
2. Ash value.
3. Extractive value.
4. pH.
5. Swelling Index.

Determination of antioxidant activity by DPPH method

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) test was used to determine the extract's ability to scavenge free radicals in vitro. It was tested by adding 1 ml of a 0.3 mM DPPH solution made in 100 percent ethanol to 3 ml of the fraction dissolved in ethanol at various concentrations. After the mixture was mixed and left to remain at room temperature for 30 minutes, the absorbance was measured using a Shimandzu spectrophotometer at 517 nm. The % scavenging activity was measured at various dosages and compared to the standard antioxidant Vitamin C. (Ascorbic acid).

$$\%\ RSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

DPPH is a stable free radical capable of accepting an electron or a hydrogen radical in order to generate a stable diamagnetic molecule. Due to its odd electron, the ethanolic solution of DPPH has a pronounced absorption band at 517nm. The electrons couple and the solution loses its colour stoichometrically as the amount of electrons taken up increases when DPPH radicals react with appropriate reducing agents. This reactivity has been used to examine the ability of compounds/plant extracts to act as free radical scavengers. The DPPH radicals have been decreased, as seen by the lower absorbance at 517nm. The IC50 was calculated using linear regression analysis. The results are shown in Table 3.

III. RESULTS AND DISCUSSION

In vitro antioxidant assay of the polyherbal compound (combination) revealed the presence of antioxidant potential. The percentage of inhibition was observed that free radicals were scavenged by the test compounds in a concentrated manner in the methods. Table 1 depicts, the results of phytochemical analysis were observed that the presence of flavonoids, phenols, tannins, alkaloids, steroids, and carbohydrates and Table 2 shows the physical evaluation of individual and combination extract.

Table 1: Physical evaluation of individual and combination of extract

Sr. No.	Parameters	Emblica officinalis	Eugenia jambolana	Terminalia chebula	Acacia catechu
1.	Moisture content	7.47±0.060	4.47±0.047	7.61±0.061	10.19±0.458
2.	Total ash	8.6±0.163	8.29±0.298	7.87±0.265	2.3±0.244
3.	Acid insoluble ash	0.73±0.120	1.33±0.124	3.66±0.132	0.44±0.036
4.	Water-soluble ash	6.46±0.124	3.39±0.147	4.31±0.177	0.29±0.041
5.	Water-soluble extractives	25.08±0.716	23.12±0.656	12.97+0.448	23.74±0.451
6.	Alcohol soluble extractives	8.21±0.103	13.98±0.677	15.57±0.324	19.74±0.506
7.	pH of water extract	5.42±0.163	5.71±0.217	5.26±0.008	6.05±0.037
8.	Swelling index	4.18	4.28	3.7	5.00

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Table 2: Phytochemical results of individual and combination of extract

S. No.	Concentration (gm/ml)	Ascorbic acid (% inhibition)	Polyherbal formulation (% inhibition)
1	12.5	38.66	25.11
2	25	42.87	45.68
3	50	62.97	63.79
4	100	65.19	69.39
5	200	67.52	75.70
6	400	70.56	81.54
IC 50 value		11.66	37.50

Table 3: DPPH activity of polyherbal formulation with reference to ascorbic acid

Sr. No.	Chemical Test	Emblica officinalis	Eugenia jambolana	Terminalia chebula	Acacia catechu
1.	Alkaloid	+	+	+	+
2.	Carbohydrates	+	+	+	+
3.	Glycosides	+	-	-	+
4.	Flavonoids	+	+	+	+
5.	Proteins	-	+	+	-
6.	Tannins	-	+	+	+
7.	Amino acids	+	+	+	-
8.	Saponins	+	+	+	+
9.	Steroids	+	+	+	-
10.	Resins	-	-	-	-

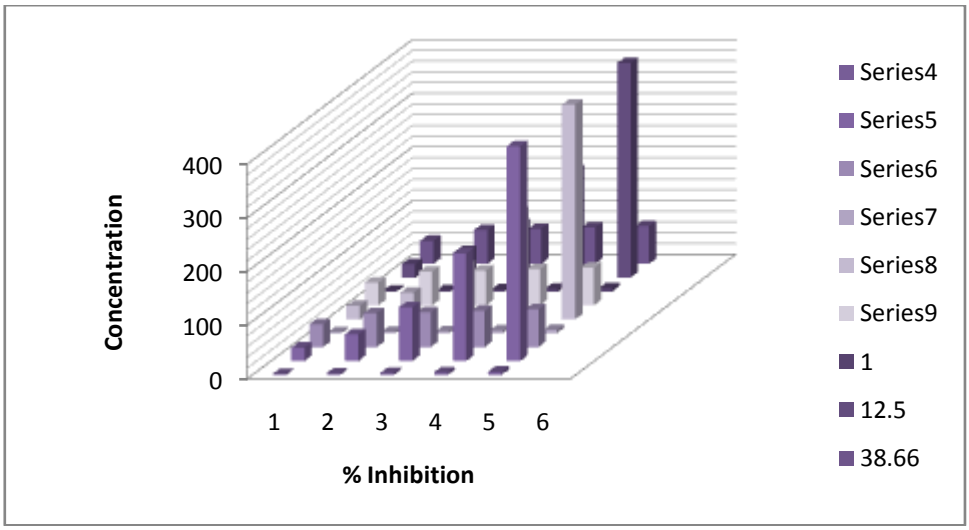

Fig.1. Antioxidant activity of Polyherbal formulation

The DPPH scavenging activities were recorded in terms of percentage inhibition observed from Table 3 that the combination has maximum DPPH. The results obtained were compared to standard ascorbic acid. Higher the percentage inhibition indicates better scavenging activity or antioxidant potential. The results obtained were statistically significant with $p<0.05$.

IV. CONCLUSION

The result obtained from above study indicates the presence of flavonoids, steroids, glycosides, and tannins in the PHF. The antioxidant

screening done using DPPH method showed a good antioxidant potential as compared to reference standard drug. From the above study, we can conclude that PHF possesses promising antioxidant activity which can be considered as a base for further pharmacological evaluation.

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