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Phytochemical Screening and In-Vitro Antioxidant Activity of An Herbal Drug 'Ashwagandharishta', Estimation of Total Phenolic and Flavonoid Contents

R. K. Pawar*[1], Dr. G. R. Bhagure[2]

[1*]Department of Chemistry M.S.G. Arts Science Commerce College Malegaon, Nasik, Maharashtra, India

[2]Department of Chemistry, Satish Pradhan Dnyanasadhana College, Thane, Maharashtra, India

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ABSTRACT

Because of their ability to neutralise free radicals, phenolic compounds have a variety of biological effects, including anti-inflammatory, antibacterial, and antioxidant characteristics. Antioxidants are essential nutrients that protect the body from the harmful effects of oxidative stress caused by free radicals. A wide range of free radical scavenging antioxidants can be found in a variety of foods. The study's goal was to find out how much total phenolic content, total flavonoids content, antioxidant activity, and phytochemical screening of herbal drugs. Flavonoids are a type of natural polyphenolic chemicals that humans are unable to produce. These compounds have a variety of biological characteristics and act as antioxidants in biological systems. The Folin-Ciocalteau method was used to determine the total phenolic content. Antioxidant properties were determined using 1,1-diphenyl-2- picrylhydrazyl (DPPH). The total phenolic and flavonoid content was determined spectrophotometrically using gallic acid and quercitin as standards. The Herbal drug for the study is purchased from the local markets of Nashik (Maharashtra). 5 percent, 2.5 percent, 1.25 percent, 0.625 percent, 0.3125 percent, and 0.15625 percent concentration samples in water of the herbal medication were prepared for the assessment of antioxidant potential by DPPH assay. As the concentration % rises, so does the antioxidant activity, as well as the overall phenolic and falvonoid content increases. The antioxidant activity is due to the existence of total phenolic and flavonoid content. Conventional methods were used to determine the presence of specific phytochemicals.

Keywords : Antioxidant, Phenolic, Flavonoids, DPPH.

I. INTRODUCTION

For millennia, medicinal plants have been used as a source of pharmacological ingredients for the prevention and treatment of diseases and afflictions. According to the World Health Organization, more than 80% of the population in poor nations uses herbal

and traditional medicines to treat common disorders. [1]

The fundamental cause of food deterioration is the oxidative processes that occur in it. They are to blame for the loss of nutritional value, as well as the deterioration of scent, taste, and texture. Furthermore, by interacting with critical for organism function molecules, the results of biological substance oxidation can disrupt cell homeostasis and behave cytotoxically, resulting in disorders such as tumours, heart failure, cataracts, and brain dysfunction. [2]. Antioxidants are chemicals that can prevent or suppress oxidation processes in both the human body and food. Natural antioxidants are found in practically all edible plant items, making them a stable aspect of nutrition. Polyphenols are the most common type of antioxidant found in fruits and vegetables, as well as their derivatives, leguminous plants, cereals, teas, herbs, spices, and wines. [3, 4]. The importance and use of antioxidants has increased as a result of the increased consumption of foods high in polyunsaturated fatty acids.

Antioxidant supplementation is a well acknowledged method of extending the shelf life and stability of food goods, especially those containing fat. However, because of their possible cancerogenicity, artificial antioxidant chemicals such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) have a limited dietary intake. [5].

Natural antioxidants are in high demand in the food and cosmetics industries, which necessitates the search for new sources of these chemicals. Numerous scientific studies point to fruit waste parts, such as seeds and peels, as being excellent sources of antioxidants, but only a few of them include waste parts of fruits, such as seeds and peels. Fruit and vegetable wastes and by-products, which are produced in large quantities during industrial processing, pose a significant concern because they have an environmental impact and must be controlled and/or exploited. They are, on the other hand, high in bioactive components, which are thought to have a positive impact on health. Efforts to enhance methods and strategies of reusing fruits and vegetable wastes have been made throughout the past decade. The main goal is to increase the value of antioxidants and other biocomponents found herbal medicine sectors. It was attempted to examine which herbal product can be considered a rich source of natural antioxidants.

The competition between natural and synthetic antioxidants, in terms of consumer acceptance, legal needs for market access, toxicity and thermal stability, is a big problem. The objective of the investigation performed was to determine the antioxidant properties of herbal drugs that are commonly available and readily consumed in India, and to indicate which of them can become a new source of natural antioxidants for the needy people. Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess herbal compounds [6]. The majority of the antioxidant capacity of a herbal compound may be from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or b-carotene [7 ,8]. Many of these phytochemicals may help to protect cells against the oxidative damage caused by free radicals [9]. Herbal antioxidants are effective in lowering the risk of degenerative diseases like cardiovascular disease, cancer, and neurological disorders [6]. There are about 5000 plant phenolics that have been identified, and model studies have shown that many of these exhibit antioxidant action [10]. The redox characteristics of phenolics, which allow them to operate as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, are primarily responsible for their antioxidant action [11]. The amount and placement of hydroxyl groups, as well as the existence of a 2-3 double bond and 4-oxofunction, are all factors that influence their antioxidant activity[12]. Flavones,

flavonols, flavonones, isoflavones, flavan-3-ols, and anthocyanins are all members of the flavonoids family of low molecular weight polyphenolic chemicals[13]. Although flavonoids are typically thought to be non-nutritive, interest in them has grown as a result of their potential health benefits [14]. Flavonoids inhibit tumor-related enzymes such as prostaglandin synthase, lipoxygenase, and cycloxygenase, and may stimulate detoxification enzymes such as glutathione Stransferase, in addition to their antioxidant properties [15].

Consumers that ingest herbal and other physiologically active ingredients enjoy longer and healthier lives. Because free radicals can harm numerous biological substances, including lipids, proteins, carbohydrates, and vitamins, the antioxidant action of several herbal drugs lessens the risk of certain diseases [16].

The low disease incidence in some countries drew attention to the nutrition of its citizens. Eskimos, for example, eat a sea-food-based diet (which is high in omega 3 and 6 lipids) yet have a low rate of heart disease. The same trend may be seen in the French who drink red wine, as well as Asian people who eat soybean and have a lower risk of breast cancer. It has been proven that eating fruits, vegetables, and certain herbal medicines reduces the risk of coronary artery disease and cancer [17].

Phenolic chemicals are abundant in herbal medicines and are an important part of the human diet. They have antioxidant, anti-aging, anti-cancer, and anti-inflammatory effects, among others [18]. Antioxidants, such as phenolic substances, primarily inhibit free radicals and prevent their chain reactions [19].

Free radicals have been linked to a variety of disorders, including tissue damage, arteriosclerosis, rheumatoid arthritis, ageing, cardiovascular diseases, neurological diseases, DNA change, cancer, and hundreds of other diseases[20]. Many intrinsic systems exist to offset the harm caused by free radicals, such as those created by the loss or addition of a single electron to a non-radical molecule, due to the enormous generation of free radicals during metabolic activities. The amounts of these free radicals vary depending on the situation, such as increased tissue oxygenation, lactic acid buildup (lactoacidosis), and ketones (ketoacidose). In most cases, our bodies fight these radicals on their own. Our systems are overburdened when radical levels grow to high levels or rise too quickly (perhaps owing to external factors), and here is where herbal medications with antioxidant capabilities come into play, helping to counteract the excess of free radicals [21]-[23]. As a result, oxidative stress occurs whenever free radicals rise and natural antioxidants fall, resulting in tissue damage. This process usually results in a direct or indirect inflammatory response, which elevates the level of free radicals and keeps tissue damage going. Dietary antioxidants play a significant role in illness prevention, and there is a direct link between oxidative stress and food, which is critical for the organism's antioxidant defence[24].

Flavonoids are a type of plant pigment that can be found in a variety of fruits and vegetables. This class of naturally occurring polyphenolic chemicals that humans cannot create has a number of biological features that operate as antioxidants in biological systems. Flavonoids impact capillary permeability and act as exogenous antioxidants, making them antiviral, anti-inflammatory, and antitumoral drugs. Flavonoids block many enzymes (ciclo-oxigenase, lipooxigenase, NADPH-oxidase, xantine-oxidase, fosfolipase) and stimulate enzymes with antioxidant activity, capturing and neutralising oxidative agents and quenching free radicals (such as catalase and superoxide dismutase). As a result, flavonoids directly interfere with the generation and propagation of free radicals[19] [22].

Flavonoids in the diet have been linked to a lower risk of arthrosclerosis, teratogenicity, and coronary disease, according to various studies. Flavonoids reduce total

blood cholesterol levels and have an antioxidant effect, which reduces the risk of arthrosclerosis, teratogenicity, and coronary disease[25].

The goal of this study was to assess the antioxidant qualities of herbal drugs by calculating total flavonoids, total phenolic content, and phytochemical screening in order to determine which herbal products can aid in maintaining a healthy lifestyle.

Materials and Method:

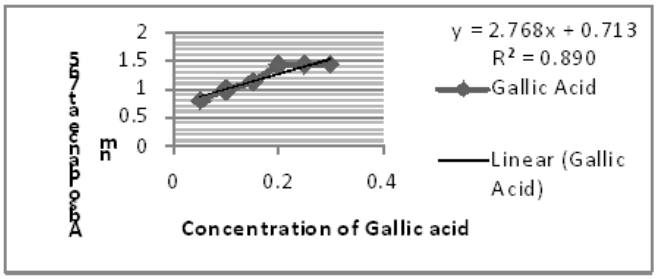
Sigma Aldrich provided all of the chemicals for the investigation, which were of excellent quality and purity. 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, gallic acid, L-ascorbic acid, and the Folin-Ciocalteu reagent are some of the reagents used. All of the chemicals utilised were of the highest quality. Sandu's herbal medicine, 'Ashwagandharishta,' was acquired from Nashik's local herbal medical stores (Maharashtra).

DPPH Assay:

The 1,1-diphenyl-2-picrylhydrazyn radical scavenging method was used to determine the free radical scavenging activity of the test samples, as described in the literature. [26]. In 100% methanol, the DPPH reagent was produced at a concentration of 0.1mM. The test samples were made with various concentrations of distilled water: 5%, 2.5 percent, 1.25 percent, 0.625 percent, 3.125 percent, and 0.15625 percent. A 0.5mL sample of the test concentration was incubated for 30 minutes at room temperature in the dark with a 0.5mL DPPH solution. After the incubation period, the absorbance was measured spectrophotometrically at 517 nm. To make acceptable colour blanks, 0.5 mL test concentration and 0.5 mL methanol were employed. As a zero control, 0.5 mL DPPH reagent and 0.5 mL methanol were employed.

Total Phenolic content :

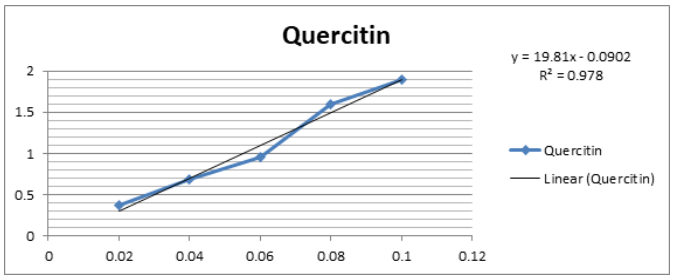
The total phenolic content was determined using the Folin-Ciocaltue reagent [27]. The extract was made in Methanol at a concentration of 1 mg/mL, and 0.2 mL was combined with 0.8 mL Folin-Ciocalteu reagent. 2.0 mL 7.5 percent Na2CO3 and 7 mL distilled water were added in total. A calibration curve was produced using gallic acid (0.05-0.3 mg/ml) as the standard. All of the tubes were incubated in the dark for 2 hours. A UV-Vis spectrophotometer set at 765 nm was used to measure the absorption. For the calibration curve on y-axis, absorbance values of Gallic acid dilutions were plotted, while concentration was plotted on the X-axis. (y=2.768x + 0.7138) was discovered to be the regression line equation. The absorbance of the sample was substituted in the equation to generate a concentration equal to the concentration of Gallic acid from the graph.



Total Flavonoids content:

A modified version of the Aluminum Chloride colorimetric approach was used to determine the total flavonoid content [28]. 500 µL of plant extract (1 mg/mL) was added to 1.5 mL of AlCL3. The blank was made with 500 µL of distilled water instead of plant extract. Quercitin (20-100 g/mL) was used as the standard. The absorbance was measured at 420 nm after 60 minutes of incubation at room temperature. Using y = 0.018x -0.094 b, where x is the absorbance and y is the concentration (mg QE) of the methanolic quercetin solutions, the total flavonoid content was determined as quercetin equivalent (QE).

The total flavonoid content was calculated using the graph.



Phytochemical Screening:

The herbal medicine was tested for the following phytochemicals[27]. All of the extracts were utilised directly in each of the following experiments (as is). The samples were analysed qualitatively for the presence of flavonoids, saponins, cardiac glycosides, terpenoids, tannins, phenols, and other active chemical components using known techniques.

Test for flavonoids:

A few drops of NaOH were added to 2 mg of extract to generate a vivid yellow colour, which decolorized even more when a few drops of strong HCl were added, indicating the presence of flavonoids.

Test for saponins (Foam Test):

2mg of extracts were mixed with 5mL distilled water and agitated to produce foam, which indicates the presence of saponins.

Test for cardiac glycosides:

2 milligrammes of extracts were treated with 2 mL glacial acetic acid containing a drop of FeCl3 solution. 1 mL pure H2SO4 was placed underneath. Cardenolide lacks de-oxy sugar characteristics because to the lack of a brown ring at the interface.

Test for terpenoids:

2 mg of extracts were treated with 2 mL chloroform, followed by a thin layer of saturated H2SO4. A reddish brown colour development at the touch confirms the presence of terpenoids.

Test for tannins:

2mg extracts were cooked for 5-10 minutes in 2mL water before being filtered. The absence of a brownish green or blue black coloration indicated the absence of tannins when ferric chloride (0.1 percent) was added.

Test for phenols (Ferric Chloride Test):

3-4 drops of FeCl3 were applied to 10mg of extracts, and the presence of bluish black precipitate was checked.

II. RESULTS AND DISCUSSION

Antioxidant activity by DPPH:

The DPPH technique was used to conduct the antioxidant experiment in vitro. Using this method, several natural product extracts were assessed for their free radical scavenging abilities. [26,29,30]. The samples had a considerable free radical scavenging effect, which increased with increasing concentration, according to the findings.

The DPPH assay evaluates antioxidants' capability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH), a non-common radical in biological systems. The table below shows the proportion of DPPH scavenging activity of herbal treatment samples at varied concentrations. The results are statistically significant; all concentrations show considerable DPPH scavenging activity, which varies from 20.123 to 96.199 percent depending on the concentration. A high degree of antioxidant capacity is indicated by a high level of scavenging activity. The reaction of the free radical DPPH with the phenolic component of the herbal medication is most likely to blame for the high percentage of scavenging activity.

Previous study has revealed that the presence of phenolic components in herbal medications may be responsible for their DPPH scavenging effect. Despite this, flavonoid's significance in scavenging action was not mentioned. The percentage of DPPH scavenging action reduces gradually as the time period lengthens. The drop in total phenolics and other components such as anthocyanins, carotenoids, and flavonoids that occurs during storage can be linked to a loss in

antioxidant activity. Because of the presence of hydroxyl functional groups, which have redox properties, it has been claimed that the antioxidant action of these drugs may be linked to the presence of phenolic substances, particularly flavonoids[31].

Zero percent antioxidant activity is defined as the optical density of the zero control. In comparison to the control, the test substance's percent antioxidant potential is calculated.

% Antioxidant potential = 100 – [Absorbance of test – Absorbance of colour blank / Absorbance of zero control]

Among the samples examined, the extract with the lowest concentration that exhibited 50% antioxidant activity was shown to be the most effective antioxidant.

Table 1 : DPPH assay showing mean antioxidant activity of extracts of *Ashwagandharishta,* the herbal medicine

Concentration %	Initial analysis	4 month Analysis	8 month Analysis	12 month analysis	16 month analysis
5	96.199	79.744	86.311	84.120	83.051
2.5	86.623	82.764	79.347	75.560	57.176
1.25	60.230	60.027	59.597	55.726	44.528
0.625	48.028	46.004	42.780	36.525	33.451
0.3125	33.311	31.505	30.120	22.807	20.123

Total phenolic and total flavonoids content:

Table- 2 : Total Phenolic and Total Flavonoids content in herbal medicine *Ashwagandharishta*.

	Initial analysis	4 month Analysis	8 month analysis	12-month analysis	16 month Analysis
Total phenolic content %	38.25	37.56	36.98	35.59	35.20
Total flavonoids content %	15.03	14.96	14.66	14.33	13.25

The presence of phenolic and flavonoids components is primarily responsible for antioxidant activity, as evidenced by the percentage of total phenolic and flavonoids content.

Phytochemicals :

The phytochemical screening of the herbal medication is done qualitatively according to the process outlined above, and the findings are shown in the table below.

Table-3 : Phytochemicals present in the herbal medicine *Ashwagandharishta*

Name of the phytochemicals	+ = present / - = absent
Saponins	+
Flavonoids	+
Phenols	+
Terpenoids	+
Cardiac Glycosides	-
Tanins	-

III. CONCLUSION

The herbal treatment 'Ashwagandharishta', according to the findings of this study, contains natural antioxidants that can scavenge free radicals. The amount of phenolic and flavonoid compounds in the chemical may influence its ability to scavenge free radicals. The results of a long-term study show that the composition of phytochemicals does not change, assisting in the preservation of the herbal medicine Ashwagandharishta's natural qualities.

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