# TOTAL PHENOLIC AND FLAVONOID CONTENTS, PHYTOCHEMICAL SCREENING, AND IN-VITRO ANTIOXIDANT ACTIVITY OF ASHOKARISHTA A HERBAL MEDICINE

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**ABSTRACT:**

'Ashokarishta' is a herbal remedy that has been utilised for centuries. Ayurvedic preparation Ashokarishta, also known as Asokaristam, is well-known. It's frequently used to address a variety of women's health concerns. The goal of this study was to assess the herbal medicine's antioxidant activity, total phenolic content, total flavonoids content, and phytochemical screening in order to determine its long-term durability. The herbal remedy was evaluated over a two-year period at four-month intervals. Using gallic acid and quercitin as standards, the total phenolic and flavonoid contents were measured spectrophotometrically. Traditional methods were used to screen for phytochemicals such as saponins, flavonoids, phenols, terpenoids, cardiac glycosides, and tannins. Free radical scavenging activity against 1,1-diphenyl-2-picrylhydrzyl was used to assess antioxidant activity in vitro (DPPH). The samples were generated in distilled water at five different concentrations: 5%, 2.5 percent, 1.25 percent, 0.625 percent, 0.3125 percent, and 0.15625 percent for antioxidant activity. The antioxidant activity of the herbal medication is strongest at greater concentrations and lowest at lower concentrations. The antioxidant activity varies between 95.980 and 26.478 percent. The most efficient antioxidant from the offered samples was found to be the lowest concentration of extract that gave 50 percent antioxidant activity. The total phenolic content varies between 71.35 and 58.65 percent. The total flavonoids content varies between 33.02 and 29.33 percent.

**Keywords :** Antioxidant**,** herbal, phenolic, flavonoids, DPPH.

## INTRODUCTION:

Herbal remedies have gained the attention of scientists in recent years as the public's interest in human health has grown. Plant secondary metabolites known as phenolic compounds have been investigated extensively and are widely used as antioxidants in a variety of applications.

Natural resources of possible biodynamic chemicals that can be employed in medicine development are medicinal plants [1]. Secondary metabolites generated from medicinal plants, such as alkaloids, flavonoids, phenols, quinones, tannins, and terpenoids, are used to treat a variety of disorders around the world. Medicinal and aromatic plants' quality and therapeutic efficacy appear to be influenced by secondary metabolites, which are influenced by environmental conditions [2]. Antioxidants are essential nutrients that protect the body from the harmful effects of oxidative stress caused by free radicals [3]. The production of free radicals is a normal part of the biological process, although it increases dramatically during pathological situations [4]. Excess free radicals can damage cellular lipids, proteins, or DNA, impairing their normal function and perhaps speeding up the ageing process [5]. Plant-derived secondary metabolites, particularly phenolic chemicals, constitute the largest group of exogenic antioxidants [6]. The

characterisation of main phenolic components of naturally occurring phytochemicals as antioxidants has been enabled by studies on the free radical-scavenging abilities of flavonoids [7].

Numerous studies have been conducted on a variety of herbal medicines to determine their natural antioxidant activity. They can act as natural antioxidants that can be used in place of synthetic ones, interfering with the oxidation process and the change of lipids, proteins, and DNA [8]. By blocking and scavenging free radical action, natural antioxidants can also provide protection against degenerative diseases, including cardiovascular disease and cancer [9].

Oxidative stress occurs when the balance between the generation of reactive oxygen and nitrogen species (ROS and RNS) in a living cell is disrupted, resulting in damage to cell components such as proteins, lipids, and nucleic acids, and ultimately cell death[10]. As a result of normal cellular metabolism, reactive oxygen species (ROS) such as hydrogen peroxide and hypochlorous acid, as well as free radicals such as the hydroxyl radical and superoxide anion, are formed. Endogenous ROS are created by mitochondria during the respiratory chain reaction whereas external ROS are formed by a variety of causes such as UV light, contaminants, and inflammation[11]. Rapid generation of free radicals can result in oxidative damage to biomolecules, which can result in diseases like cancer, diabetes, inflammatory disease, asthma, cardiovascular disease, neurological disease, and premature aging[12]. ROS and RNS are the primary sources of free radicals and are involved in the pathogenesis of neurological disorders such as Alzheimer's disease, Parkinson's disease, and strokes[13-15]. Antioxidants protect against damage caused by uncontrolled production of reactive oxygen species (ROS) and associated lipid peroxidation, protein damage, and deoxyribonucleic acid strand breakage[16]. The ideal antioxidant should be readily absorbed, neutralise free radicals, and chelate metal redox at physiologically relevant concentrations [17].

The human body does have a complex antioxidant defence system that includes antioxidant enzymes such as superoxide dismutase, glutathione 2 peroxidase, and catalase, as well as non-enzymatic antioxidants such as glutathione[18], vitamins E and C, thiol antioxidants (glutathione, thioredoxin, and lipoic acid), melatonin, and carotenoids[17]. Numerous adverse effects have been documented with currently available synthetic antioxidants, including butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone, and gallic acid esters [19,20]. As a result, research has concentrated on naturally occurring chemicals. Plant phytochemicals not only protect against oxidative stress caused by free radicals, but also mitigate the adverse effects of synthetic antioxidants.

Herbal medicine is one of the most important branches of traditional medicine around the world, and the use of herbal treatments for a variety of medical ailments is becoming increasingly popular. Plant phytochemical components are increasingly being linked to their medicinal activities[21]. Natural antioxidants derived from medicinal plants have been shown in recent studies to protect against the toxic and harmful effects of free radicals and to have a wide range of pharmacological effects, including antimicrobial, antimutagenic, antiallergic, antioxidant free radical scavenging activity, and anticarcinogenic effects [22-24].

Nature has provided food, housing, medicine, and other resources to people since the beginning of time [25]. The importance of herbal medicine to human health and the interrelationship between society and nature has recently gained widespread recognition, drawing attention to the fact that loss of biodiversity, destruction, or unscientific use of medicinal plants can have direct and indirect effects on human well-being. Our ecosystem is becoming less robust and more prone to shocks and disturbances as biodiversity is disrupted and forest and plant resources are depleted. Human health cannot be considered in isolation because it is highly dependent on the quality of the environment in which we live; in order for people to be healthy, they require healthy environments as well as a proper medical care system that provides environmentally friendly, bio-friendly, cost-effective, and relatively safe treatments [25-27].

Medicinal herbs are becoming more popular in mainstream healthcare in the twenty-first century, as more individuals seek relatively safe therapies and approaches to healthcare. The global demand for herbal medicines, herbal health products, herbal pharmaceuticals, nutraceuticals, food supplements, and herbal cosmetics, among other products, is growing due to a growing recognition of these products as primarily non-toxic, with fewer side effects, better compatibility with physiological flora, and affordability [28,29]. Traditional therapeutic systems include medicinal plants as a key component. Herbal remedies have been utilised and documented in Indian, Chinese, Egyptian, Greek, and Roman medicinal systems for over 5000 years, according to the earliest documents. Traditional herbal medicine has also been practised in America, Arabia, and Japan since ancient times. Rigveda, Atherveda, Charak Samhita, and Sushruta Samhita are

examples of India's classical traditional medical systems. The indigenous healthcare system relies heavily on folk (tribal) remedies.

Education and training in herbal medicine face significant challenges. The first problem is to ensure that conventional medicine providers have the necessary knowledge, skills, and training. It is critical to provide training to ensure that traditional medicine providers and allopathic practitioners understand and respect the complementary nature of this type of healthcare. Although governments recognise it to varied degrees, the absence of solid scientific evidence of the efficiency of many traditional herbal medicine systems, difficulties with the safeguarding of indigenous traditional knowledge, and issues with assuring its proper use must be addressed promptly. Intellectual property problems should also be addressed in order to prevent biopiracy of indigenous traditional knowledge and/or natural resources used in traditional medical goods [30].

The primary significant criterion in justifying herbal formulations and crude medications' adoption in modern medicine is quality control/standardization. Good Manufacturing Practices (GMP) are urgently needed to assure the excellent quality of herbal medications. To meet the public's high expectations, a drug regulatory body must conduct well-designed, randomised, double-blind, placebo-controlled clinical trials to establish the safety and efficacy of herbal medicines in conjunction with allopathic pharmaceuticals. Systematic clinical trials will open up new avenues for basic and applied research on herbal items and Ayurvedic treatments utilised in India. The Indian Pharmacopoeia 2007 featured 59 monographs on herbs and herbal Products, while the Indian Pharmacopoeia 2010 included 89 [31,32]. GMP for Ayurvedic, Siddha, and Unani medicine became mandatory in the year 2000. The Ayurvedic Pharmacopoeia of India contained 258 distinct medications in 2005, and the Indian Herbal Pharmacopoeia contained 52 monographs of commonly used medicinal plants [33].

We chose Ashokarishta, a herbal remedy, for the investigation. This herbal medication was tested for antioxidant activity using the DPPH assay, total phenolic and total flavonoids, and phytochemical screening.

## MATERIALS AND METHODS:

Sigma Aldrich provided the chemicals utilised in the investigation. Folin-Ciocalteu reagent, quercetin, gallic acid, L-ascorbic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH). All of the chemicals utilised were of analytical quality. Baidyanath's natural remedy Ashokarishta was purchased in Nashik's local marketplaces (Maharashtra).

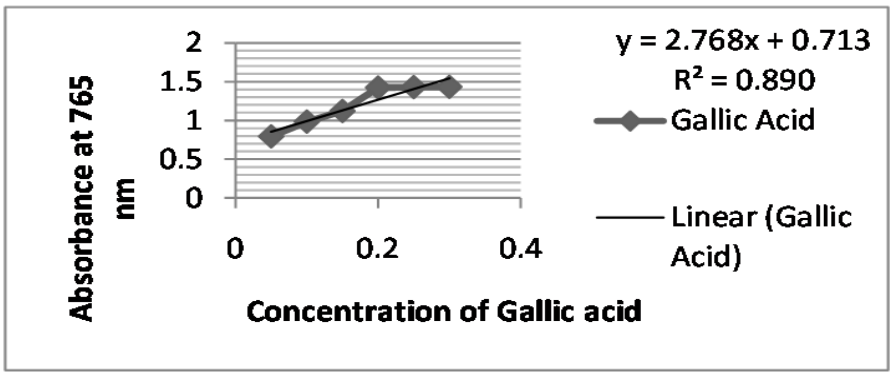
### DPPH Assay:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method was employed to determine the free radical scavenging activity of the test material, as previously reported[34]. At a concentration of 0.1mM, the DPPH reagent was prepared in 100 percent methanol. Test samples were obtained at concentrations of 5%, 2.5 percent, 1.25 percent, 0.625 percent, 3.125 percent, and 0.15625 percent in distilled water. Incubation of a 0.5mL sample of the test concentration with a 0.5mL DPPH solution. The mixture was incubated at room temperature in the dark for 30 minutes. After incubation, the absorbance at 517 nm was determined spectrophotometrically. To prepare 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 [35]. The extract was prepared at a concentration of 1 mg/mL in Methanol 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 as the standard (0.05-0.3 mg/ml). All tubes were incubated in the dark for 2 hours. The absorbance was determined at 765 nm using a UV-Vis spectrophotometer. The calibration curve was constructed by graphing the absorbance readings of Gallic acid dilutions on the y-axis against the concentration on the x-axis. (y=2.768x + 0.7138) was determined to be the regression line equation. The

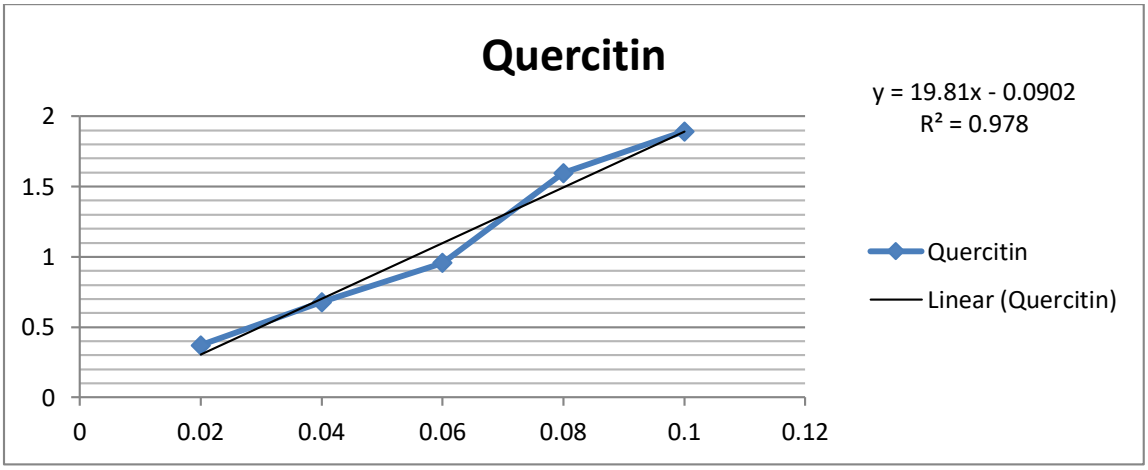
absorbance of the sample was replaced into the equation to obtain a concentration equal to that of Gallic acid as determined by the graph.




**Total Flavonoids content:**

A modified version of the Aluminum Chloride colorimetric technique was used to determine the total flavonoid content [36]. To 1.5 mL of AlCL3, 500 mL of plant extract (1 mg/mL) was added. The blank was prepared using 500 litres of distilled water rather than plant extract. Quercitin (20–100 g/mL) was used as a standard. After 60 minutes of incubation at room temperature, the absorbance at 420 nm was determined. The total flavonoid content was calculated as quercetin equivalent (QE) using the equation y = 0.018x -0.094 b, where x is the absorbance and y is the concentration of the methanolic quercetin solutions (mg QE).

The graph was used to determine the flavonoid content in total.




**Phytochemical Screening:**

The herbal medication was evaluated for the presence of the following phytochemicals[35]. All extracts were utilised directly in each of the subsequent tests (as is). The samples were qualitatively analysed for the presence of several kinds of active chemical components, including flavonoids, saponins, cardiac glycosides, terpenoids, tannins, and phenols, among others.

**Test for flavonoids:** A few drops of NaOH were applied to 2 mg of extract to produce a bright yellow colour, which decolorizes further when a few drops of concentrated HCl is added, confirming the presence of flavonoids.

**Test for saponins (Foam Test):** 5mL of distilled water was added to 2mg of extracts and shaken for the production of froth, which shows the presence of saponins.

**Test for cardiac glycosides:** 2 mL glacial acetic acid containing a drop of $FeCl_3$ solution was used to treat 2 milligramme of extracts. This was under layered with 1 mL of concentrated $H_2SO_4$. The absence of a brown ring at the interface suggests that cardenolide lacks de-oxy sugar properties.

**Test for terpenoids:** 2 mg of extracts were treated with 2 mL of chloroform, then a layer of concentrated $H_2SO_4$ was carefully applied. The presence of terpenoids is confirmed by a reddish brown colour development at the contact.

**Test for tannins:** 2mg of extracts were boiled in 2mL water for 5-10 minutes before being filtered. Ferric chloride (0.1%) was added to this, and the absence of a brownish green or blue black colouring confirmed the absence of tannins.

**Test for phenols (Ferric Chloride Test):** 3-4 drops of $FeCl_3$ were applied to 10mg of extracts, and the presence of bluish black precipitate was checked.

**Results and Discussion:**

**Antioxidant activity by DPPH:**

The antioxidant assay was conducted in vitro using the DPPH method. Several natural product extracts have been assessed using this method for their free radical scavenging activity [34,37,38]. According to the findings, the samples demonstrated a substantial ability to scavenge free radicals that increased with concentration.

The DPPH assay is used to determine an antioxidant's ability to decrease 1,1-diphenyl-2-picrylhydrazyl (DPPH), another radical found in biological systems. The table below summarises the percentage of DPPH scavenging activity of herbal treatment samples at varied concentrations. The reported results are statistically significant; all concentrations exhibit a significant amount of DPPH scavenging activity, ranging from 76.3 to 97.879 percent at various doses. Scavenging activity is a good indicator of antioxidant capability. The high scavenging activity is most likely due to the reactivity of the free radical DPPH with the herbal medicine's phenolic component.

According to previous study, the DPPH-scavenging activity of herbal medicines may be due to the presence of phenolic components. Despite this, no mention was made of the involvement of flavonoids in scavenging activities. The percentage of DPPH scavenging action reduces gradually as the time period lengthens. The decrease in antioxidant activity associated with storage can be attributed to a decrease in total phenolics and other components such as anthocyanins, carotenoids, and flavonoids. It has been claimed that these drugs' antioxidant activity is due to the presence of phenolic chemicals, particularly flavonoids, which contain hydroxyl functional groups with redox properties [39].

The optical density of the zero control is considered as zero percent antioxidant activity. Percent antioxidant potential of the test substance is calculated in comparison with the control.

% 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 *Ashokarishta*, the herbal medicine.**

| Concentration % | Initial analysis | 4 month Analysis | 8 month Analysis | 12 month analysis | 16 month Analysis |
|---|---|---|---|---|---|
| 5 | 96.564 | 94.560 | 92.940 | 91.628 | 90.468 |
| 2.5 | 95.980 | 95.923 | 82.304 | 73.010 | 72.345 |
| 1.25 | 83.096 | 78.606 | 59.600 | 59.539 | 53.972 |
| 0.625 | 56.082 | 48.904 | 46.995 | 46.514 | 46.340 |
| 0.3125 | 41.299 | 36.500 | 32.555 | 29.435 | 26.478 |

**Total phenolic and total flavonoids content:**

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

| | Initial analysis | 4 month Analysis | 8 month analysis | 12-month analysis | 16 month Analysis |
|---|---|---|---|---|---|
| | | | | | |
| Total phenolic content % | 71.35 | 70.96 | 69.97 | 67.03 | 58.65 |
| Total flavonoids content % | 33.02 | 32.79 | 31.78 | 31.23 | 29.33 |

The percentage of total phenolic and flavonoid content indicates that antioxidant activity is primarily a result of the presence of phenolic and flavonoid components.

**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 *Ashokarishta.***

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

**Conclusion:**

According to this study's findings, the herbal treatment Ashokarishta includes natural antioxidants capable of scavenging free radicals. The phenolic and flavonoid content of the chemical may be critical for its free radical scavenging action. The extensive analysis undertaken over time reveals that the composition of phytochemicals does not change, assisting in the maintenance of the herbal remedy Ashokarishta's natural characteristics.

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