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Estimation of Total Phenolic and Flavonoid Contents, Phytochemical Screening and *in vitro* Antioxidant Activity of Drakshasav A Herbal Drug

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ABSTRACT

The current study's goals were to investigate the herbal drug's antioxidant activity, total phenolic and total flavonoid content, stability, antibacterial activity, and phytochemical screening. In today's medical practise, there is a growing knowledge and acceptance of the use of herbal medications. In recent years, more people around the world have turned to natural drugs. Herbal drug technology is used to create medicines from botanical resources. The standardisation of herbal drugs is a key step in determining constituent antioxidant activity, total phenolic and total flavonoid content, and phytochemical screening. We purchased a herbal medicine called "Drakshasav" from Nashik's local markets for the study (Maharashtra). In vitro antioxidant activity was measured using free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazine (DPPH). Using gallic acid and quercitin as standards, the total phenolic and flavonoid content was measured spectrophotometrically. For two years, at four-month intervals, the selected herbal medication was examined for antioxidant activity as well as total phenolic and total flavonoids content. For antioxidant activity, different concentration solutions in water were made (5 percent, 2.5 percent, 1.25 percent, 0.625 percent, 0.3125 percent, and 0.15625 percent). It has increased antioxidant activity at increasing concentrations, and its antioxidant potential fluctuates with time. Using gallic acid and quercitin as standards, the total phenolic and flavonoid content was measured spectrophotometrically.

KEYWORDS: Antioxidant, Phenolic, Flavonoids, DPPH.

INTRODUCTION

Antioxidants are known to counteract the effects of free radicals, which can set off a series of events that can lead to oxidative stress and cellular damage. These free radicals are produced by cellular metabolism, and they have a deleterious impact on health, including cancer, hypertension, heart disease, and diabetes [1]. Herbal medications contain a variety of antioxidants, including phenolics, flavonoids, tannins, vitamins, quinines, coumarins, lignans, and ligins [2,3]. Flavonoids are antioxidant, anti-inflammatory, antibacterial, and antiviral chemicals [4]. Polyphenols, which aromatic compounds with hydroxyl groups directly connected to benzene, are a type of natural molecule that has a number of properties, including significant antioxidant, anti-inflammatory, antibacterial, and anti-aging properties [5].

Active substances derived from herbal medicinal sources have recently risen to the top of scientists' study agendas across the globe. Herbal medications are widely utilised in the treatment of various ailments. Herbal medicines are rich in antioxidants and bioactive compounds.

Nature's phenolic and flavonoid chemicals have an aromatic ring with at least one hydroxyl group [6]. Phenolic substances may contribute directly to antioxidant activity by increasing the production of endogenous oxidant molecules in the cell [7,8]. The capacity of phenolic compounds to suppress free radicals, breakdown peroxides, and prevent oxidative illnesses has been demonstrated in several studies [9].

In poor nations, non-conventional medicine is used by around 80% of the population for primary healthcare [10]. More people in underdeveloped nations rely on herbal and traditional medicine for treatment of diseases, owing to the paucity of contemporary health services and the relatively low cost of traditional medicines [11,12]. Flavonoids and other phenolic compounds are natural phytochemicals found in herbal remedies, which have led to their medicinal use in the treatment of many disorders [13,14]. Phenolic substances have a variety of biochemical and pharmacological properties, and their interactions with important enzymes, signalling cascades involving cytokines and transcription factors, and antioxidant systems may give health advantages [15–17].

Antioxidant effects of phenolic substances, particularly flavonoids, have been studied and proved to be useful [16,18]. Such bioactive substances may have antioxidant effects by scavenging free radicals, decreasing -tocopherol radicals, activating antioxidant enzymes, chelating metal catalysts, triggering apoptosis, and reducing oxidative stress [19,20]. In humans, oxidative stress can induce tissue destruction and contribute to

chronic degenerative illnesses like cancer [21–23]. The efficacy of bioactive substances to provide antioxidant protection in the setting of cancer is determined by their ability to inactivate, block, and lessen the cancer-promoting effect of certain oxygen radicals in inhibiting carcinogenesis development [24–26]. Cancer is one of the leading causes of morbidity and mortality worldwide, with developing nations accounting for more than half of all cancer cases [27,28]. Medicinal herbs have played a significant role in the development of modern medications that are used to treat a variety of disorders, including cancer, with natural products accounting for over 71 percent of new drugs licenced since 1981 [29,30]. Some Malawians employ herbal and traditional medicines to treat a variety of conditions, including cancer, because they are regarded to be less expensive and have less negative effects [31–33].

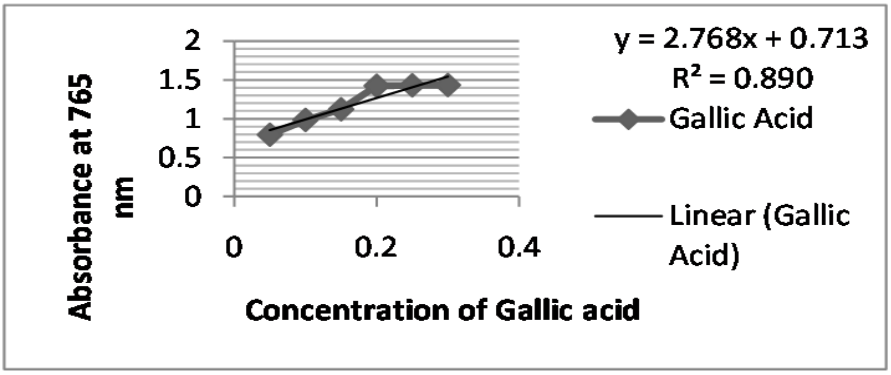
Many newly found natural compounds have an important role in preventing and treating diseases in humans, and their efficacy makes them the treatment of choice when compared to manufactured medications [34]. Free radicals, for example, are known to cause a variety of disorders, including cancer, hyperlipidemia, and hypertension [35]. Oxidative stress causes an imbalance of oxygen free radicals, which destroy organic molecules such as DNA, lipids, and proteins. Lipid hydroperoxides, isoprostan, and ubiquinol-10 are examples of oxidative stress markers. Antioxidants, such as catalase, are synthesised by human physiology to prevent cellular damage caused by free radicals. Antioxidants scavenge free radicals and repair damage by releasing hydrogen molecules or attaching themselves to the DNA of the host organism [36,37]. For the study we have selected *Drakshasav,* a herbal medicine. This herbal medicine have been analyzed for the Antioxidant activity by DPPH assay, total phenolic and total flavonoids and phytochemical screening have been done.

EXPERIMENTAL

Chemicals used in the study were purchased from Sigma Aldrich. Specifically, Folin-Ciocalteu reagent, quercetin, gallic acid, L-ascorbic acid, 1,1-diphenyl-2- picrylhydrazyl (DPPH). The chemicals used all were analytical grade. The herbal medicine *Drakshasav* manufactured by *Dhutpapeshwar* was purchased from the local markets of Nashik (Maharashtra).

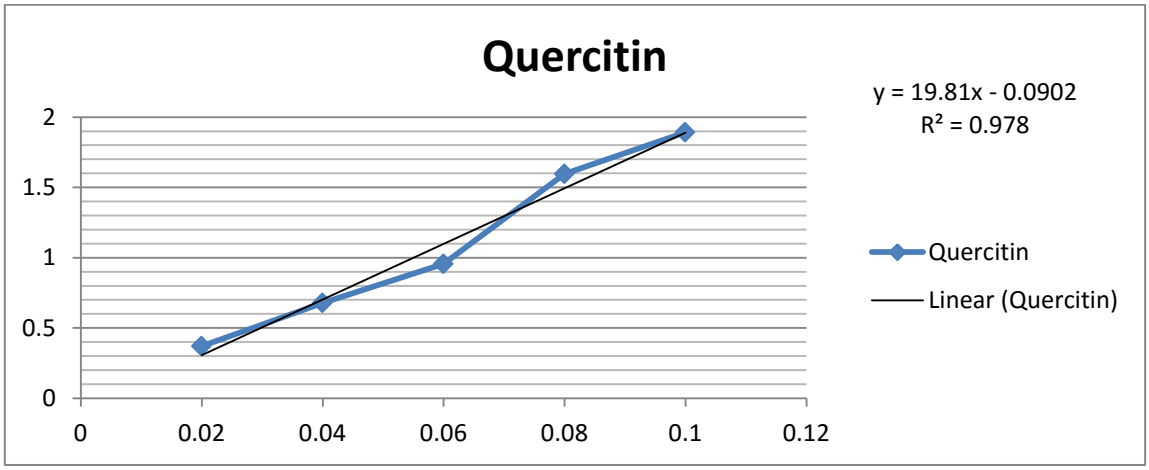
DPPH Assay: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method was used to assess the test sample's free radical scavenging activity, as reported in the literature[38]. The DPPH reagent was produced in 100 percent methanol at a concentration of 0.1mM. In distilled water, test samples were generated at concentrations of 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 with a 0.5mL DPPH solution. For 30 minutes, the mixture was incubated at room temperature in the dark. The absorbance was measured spectrophotometrically at 517 nm after the incubation period. 0.5 mL test concentration and 0.5 mL methanol were used to make appropriate colour blanks. 0.5 mL DPPH reagent and 0.5 mL methanol were used as a zero control.

Total Phenolic content: Folin-ciocaltue reagent [39] was used to determine the total phenolic content. The extract was produced at a concentration of 1mg/mL in Methanol, and 0.2 mL of this was mixed with 0.8 mL of Folin-Ciocalteu reagent. A total of 2.0 mL of 7.5 percent Na_2CO_3 and 7 mL of distilled water were added. A calibration curve was created using gallic acid (0.05-0.3 mg/ml) as the standard. For 2 hours, all of the tubes were incubated in the dark. The absorbance was measured using a UV-Vis spectrophotometer at 765 nm. The calibration curve was created by plotting absorbance values of Gallic acid dilutions on the y-axis and concentration on the X-axis. The regression line equation was found to be (y=2.768x + 0.7138). 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: The total flavonoid content was determined using a modified version of the Aluminum Chloride colorimetric technique [40]. To 1.5 mL of $AlCL_3$ 500 µL of plant extract was added (1mg/mL). Instead of plant extract, 500 µL of distilled water were used to make the blank. The standard was quercitin (20-100 g/mL). The tubes were incubated at room temperature for 60 minutes, and the absorbance was measured at 420 nm. Based on the calibration curve, the total flavonoid content was estimated as quercetin equivalent (QE) 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 graph was used to calculate the total flavonoid content.



Phytochemical Screening: The following phytochemicals were examined in the herbal drug[39]. For each of the following assays, all of the extracts were used directly (as is). Using established protocols, the samples were analysed qualitatively for the presence of numerous classes of active chemical ingredients such as flavonoids, saponins, cardiac glycosides, terpenoids, tannins, phenols, and so on.

Test for flavonoids: A few drops of NaOH were applied to 2 mg of extract to produce a bright yellow colour, whic h 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 experiment was done in vitro using the DPPH technique. Several natural product extracts have been evaluated for their free radical scavenging activities using this approach [38,41,42]. According to the findings, the samples had a significant free radical scavenging effect that increased with increasing concentration.

The DPPH assay assesses antioxidants' ability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH), another radical that is uncommon in biological systems. The percentage of DPPH scavenging activity of herbal medication samples in various concentrations is shown in the table below. The observed results are statistically significant; all concentrations have substantial DPPH scavenging activity, which ranges from 76.3 to 97.879 percent for different concentrations. A high level of scavenging activity indicates a high level of antioxidant capacity. The high percentage of scavenging activity is most likely caused by the reaction of the free radical DPPH with the phenolic content of the herbal medicine.

Previous research has suggested that the DPPH scavenging activity of herbal drugs may be attributed to the presence of phenolic components. Despite this, the role of flavonoid in scavenging activity was not highlighted. As the time period lengthens, the percentage of DPPH scavenging action decreases slightly. The reduction in antioxidant activity during storage can be related to a drop in total phenolics and other components such as anthocyanins, carotenoids, and flavonoids when the herbal medication is stored. It has been suggested that the antioxidant action of these medications may be attributed to the presence of phenolic compounds, especially flavonoids, because of the presence of hydroxyl functional groups, which have redox characteristics [43].

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.

$$\% \text{ Antioxidant potential} = 100 - [\text{Absorbance of test} - \text{Absorbance of colour blank} / \text{Absorbance of zero control}]$$

The extract with the lowest concentration that provided 50% antioxidant activity was found to be the most effective antioxidant among the samples tested.

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

Concentration %	Initial analysis	4 month Analysis	8 month Analysis	12 month analysis	16 month analysis
5	97.879	95.499	95.370	81.250	73.600
2.5	73.221	69.720	69.473	68.582	62.136
1.25	62.894	59.130	51.409	43.665	32.565
0.625	38.127	36. 018	32.690	23.733	18.605
0.3125	29.267	28.326	21.560	15.327	9.619

Total phenolic and total flavonoids content:

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

	Initial analysis	4 month Analysis	8 month analysis	12-month analysis	16 month Analysis
Total phenolic content %	39.05	38.99	38.64	37.06	32.25
Total flavonoids content %	26.42	26.11	25.89	25.63	25 .02

The percentage of total phenolic content and total flavonoids content shows that an antioxidant activity is mainly due to presence of phenolic and flavonoids compounds.

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 *Drakshasav*.

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

CONCLUSION

According to the findings of this study, the herbal remedy Drakshasav contains natural antioxidants with free radical scavenging capacity. The compound's phenolic and flavonoid concentration may play a key impact in its free radical scavenging activity. The comprehensive investigation conducted over a period of time demonstrates that the composition of phytochemicals does not change, which aids in the preservation of the original properties of the herbal medication Drakshasav.

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