**Quantitative Assessment of Polyphenols, Flavonoids, and Antioxidant Activity in Ethanol Extracts of *Artemisia absinthium* and *Acorus calamus* from Kashmir**

**ABSTRACT**

The present study aimed to quantify the total polyphenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *Artemisia absinthium* L. and *Acorus calamus* L., collected from the northern and southern regions of the Kashmir Valley. Ethanol extracts of both plant species were prepared using a magnetic stirrer-assisted maceration method. TPC was determined using the Folin–Ciocalteu assay, while TFC was assessed via the aluminum chloride colorimetric method. Antioxidant activity was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Results demonstrated that *A. absinthium* exhibited significantly higher levels of bioactive compounds, with TPC and TFC values of 128.4 mg gallic acid equivalents (GAE)/g and 18.27 mg quercetin equivalents (QE)/g dry weight, respectively. In contrast, *A. calamus* recorded lower values, with 12.02 mg GAE/g and 5.65 mg QE/g dry weight. Furthermore, *A. absinthium* exhibited a notably higher antioxidant capacity, achieving 63.40% DPPH radical scavenging activity, compared to 38.7% in *A. calamus*. These findings suggest that *A. absinthium* is a potent natural source of polyphenols and flavonoids with strong antioxidant potential. Its superior phytochemical profile highlights its promise for further exploration in pharmaceutical and nutraceutical applications, particularly in the development of natural antioxidants.

**Keywords:** *Artemisia absinthium, Acorus calamus, Polyphenols, Flavonoids, DPPH, .*

**1. INTRODUCTION**

*A. absinthium* and *A. calamus* are well-known medicinal plants traditionally used in various systems of medicine across Asia and Europe. The genus Artemisia includes more than 500 species, many of which are used in folk medicine for their anti-inflammatory, anti-malarial, and antimicrobial properties (Abad *et al.,* 2012). *Artemisia annua*, in particular, has gained global attention due to its active compound artemisinin, widely used in the treatment of malaria (Tu, 2011). Similarly, *A. calamus*, commonly known as sweet flag, has long been employed in Ayurveda and traditional Chinese medicine for its effects on digestion, cognition, and respiratory ailments (Ghosh, 2014). Both plants are reported to be rich in phytochemicals including alkaloids, terpenoids, flavonoids, and polyphenols, which are known to exert diverse biological activities (Singh *et al.,* 2016; Koffi-Nevry *et al.,* 2012).

Among these phytochemicals, polyphenols and flavonoids have garnered increasing attention due to their significant antioxidant potential, which plays a vital role in protecting cells from oxidative stress and associated diseases such as cancer, cardiovascular disorders, and neurodegeneration (Kumar & Pandey, 2013; Działo *et al.,* 2016). The antioxidant activity of plant-derived compounds is mainly attributed to their ability to donate hydrogen atoms or electrons and stabilize free radicals. In recent years, the demand for natural antioxidants has increased over synthetic alternatives, due to their safer and more biocompatible nature (Abdalla & Roozen, 1999). The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay is among the most commonly used methods for evaluating antioxidant activity due to its accuracy and reproducibility (Sanchez-Moreno, 2002).

Despite the widespread use of *A. absinthium* and *A. calamus* in traditional remedies, comprehensive studies on their comparative polyphenolic and flavonoid content and corresponding antioxidant activities are limited. Hence, the present investigation was undertaken to extract, quantify, and compare the antioxidant potential of these two medicinal plants using DPPH assay, with particular focus on their flavonoid and polyphenolic constituents.

**2. MATERIALS AND METHODS**

**2.1 Collection Site and Samples**

The samples of *A. absinthium* and *A. calamus* were collected from north Kashmir (Walur and Gulmarg) and south Kashmir (Kokernag and Achabal). The samples comprising of almost equal proportion from both and were taken from all sides of the plant by following standard procedure.

**2.2 Processing Of Samples**

The samples after collection were first washed with running tap water to decontaminate leaves from dust and other foreign materials followed by washing with distilled water. The samples were air dried on filter papers and then oven dried at 60- 65oC (Chapman, 1964) till constant weight was obtained. The samples were crushed in stainless steel blender and sieved through 2 mm mesh sieve and were stored in labeled paper envelops for subsequent analysis.

**2.3 Preparation of Extracts**

For preparation of the extract, 0.5g from each sample were dissolved in 10ml of 80% ethanol and kept at room temperature for 24 hours. Sample concentrations of 50mg/ml were diluted with 80% ethanol to make 25, 50, 100 and 200µg/ml concentrations.

**2.4 Determination of Total Polyphenolic Content (TPC)**

TPC of *A. absinthium* and *A. calamus* was determined by using the Folin–denis colorimetric method described by Kim *et al.,* (2009). About 100µl of the extracts from different concentrations (i.e, 6.25, 12.50, 25 and 50 mg/ml) were taken and mixed with 2ml of 2% Na2CO3 and100µl of 50% Folin-Ciocalteu reagent (FCR). The mixture was left for 30min to react at room temperature and absorbance was measured at 720 nm using Hitachi U-1800 UV-Vis spectrophotometer. A calibration curve of standard reference was established using gallic acid (range of concentration from 0 to 500μg/ml). TPC was revealed as gallic acid equivalents in milligrams per 100g of dry weight (mg GAE/100g DW).

**2.5 Determination of Total Flavonoid Content (TFC)**

TFC of *A. absinthium* and *A. calamus* was determined by aluminum chloride colorimetric assay. 1 ml of sample (different concentrations of 0.25, 0.50, 0.75, 1mg/ml) from each sample and 4ml of distilled water were taken in test tubes, 0.3ml of 5% NaNO2 was added and the mixture was allowed to react for 5 min at room temperature followed by addition of 0.3ml of 10% AlCl3, the mixture was again left to react for 5-6 minutes at room temperature. Further, 2ml of 1M NaOH and 2.4ml distilled water was added to each test tube to make the final volume of 10ml. Absorbance was taken at 510nm using Hitachi U-1800 UV-Vis spectrophotometer. The total flavonoid content was measured from the standard Quercetin curve (0-1mg/ml). TFC was revealed as quercetin equivalent in milligram per gram dry weight (mg QE/g DW).

**2.6 Determination of DPPH Radial Scavenging Activity**

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was analyzed by the method described by Nithiananthian *et al.,* (2011) with slight modification. Different concentrations (25, 50, 100 and 200μg/ml) of sample extracts were taken for analysis. 2ml of DPPH (0.1mM/ml or 0.004%) solution was added to 2ml of sample extracts of each concentration. In case of control, 2ml of sample extract was replaced by 80% ethanol. All the mixtures were mixed and left to incubate in dark for 30 minutes at room temperature. Absorbance was measured at 517nm on UV/VIS spectrophotometer (Hitachi U-1800) and scavenging activity percentage was calculated as follows:

I % = [(Ao – As)/Ao] x 100

Where,

Ao represent the absorbance value of the control reaction.

As represent the absorbance value of the sample extract.

I% represent the percentage inhibition.

**3. RESULTS AND DISCUSSION**

**3.1 Total Polyphenolic and Flavonoid Content**

The total polyphenolic content (TPC) and total flavonoid content (TFC) of *A. absinthium* and *A. calamus* are presented in Table 1 and Figure 1. The TPC of *A. absinthium* was significantly higher at 128.4 mg GAE/g, compared to 12.02 mg GAE/g in *A. calamus*, indicating a much richer phenolic composition in *A. absinthium*. This is consistent with earlier studies reporting high polyphenol content in *Artemisia annua* and *Artemisia vulgaris* (Ferreira *et al.,* 2010; Shah *et al.,* 2020). Polyphenols are known for their antioxidant, antimicrobial, and anti-inflammatory activities due to their redox properties (Shoib & Shahid, 2015; Soobrattee *et al.,* 2005).

The TFC also followed a similar trend, with *A. absinthium* showing 18.0 mg QE/g and *A. calamus* showing 5.65 mg QE/g. These results align with reports by Shah et al. (2020), who observed higher flavonoid concentrations in methanolic extracts of *A. absinthium* species. Flavonoids in *A. absinthium* contribute to several biological functions including antioxidant, hepatoprotective, and anti-cancer properties (Kumar & Pandey, 2013). In contrast, although *A. calamus* contains various bioactives, its comparatively lower flavonoid content may result in reduced antioxidant potential (Singh *et al.,* 2016).

**Table 1:** Total polyphenolic content (TPC) of sample extracts of *A. absinthium* and *A. calamus*. (mg GAE/g)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Concentration mg GAE /g** | | | | |
| 6.25  (mg/ml) | 12.5 (mg/ml) | 25  (mg/ml) | 50 (mg/ml) | Mean |
| *A. absinthium* | 30.8 | 110.8 | 175.5 | 196.6 | 128.4 |
| *A. calamus* | 3.3 | 10.7 | 15.6 | 18.5 | 12.02 |

**Table 2:** Total flavonoid content (TFC) of sample extracts of *A. absinthium* and *A. calamus*. (mg QE/g)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Concentration (mg QE/g)** | | | | |
| 0.25  (mg/ml) | 0.50 (mg/ml) | 0.75 (mg/ml) | 1.00 (mg/ml) | Mean |
| *A. absinthium* | 8.4 | 16.4 | 21.5 | 26.8 | 18.27 |
| *A. calamus* | 2.1 | 3.5 | 7.4 | 9.6 | 5.65 |

**Figure 1:** Graphical representation of Total polyphenolic content (TPC) and Total flavonoid content (TFC) in *A. absinthium* and *A. calamus*.

**3.2 DPPH Scavenging Activity**

The DPPH radical scavenging activity of *A. absinthium* and *A. calamus* extracts is presented in Table 3 and Figure 2. The antioxidant activity, as measured by percent inhibition of DPPH radicals, was significantly higher in *A. absinthium* (63.40%) compared to *A. calamus* (38.7%), indicating a stronger free radical neutralizing potential in *A. absinthium*. These findings are supported by previous studies, which report DPPH scavenging activity in *Artemisia annua* and *A. absinthium* ranging between 75% and 87% depending on the extract and conditions (Ferreira *et al.,* 2010; Shah *et al.,* 2020). There is slightly less antioxidant activity percentage comparing to these previous studies, this can be attributed to some environmental and geographical factors of a particular location.

In contrast, the DPPH scavenging activity of *A. calamus* has been observed to range between 30% and 42%, reflecting comparatively lower antioxidant potential (Singh *et al.,* 2016; Tiwari *et al.,* 2014). The significant difference in activity corresponds well with the total polyphenol and flavonoid content of the two plants. As antioxidants act by donating electrons to neutralize free radicals like DPPH, the higher concentration of phenolic compounds in *A. absinthium* contributes to its superior antioxidant capacity (Soobrattee *et al.,* 2005).

**Table 3:** DPPH radial scavenging activity content of sample extracts of *A. absinthium* and *A. calamus*. (%)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Scavenging (%)** | | | | |
| 25 (µg/ml) | 50 (µg/ml) | 100 (µg/ml) | 200 (µg/ml) | Mean |
| *A. absinthium* | 25.12 | 45.34 | 86.50 | 96.67 | 63.40 |
| *A. calamus* | 12.21 | 23.36 | 40.30 | 78.93 | 38.7 |

**Figure 2:** Graphical representation of DPPH radial scavenging activity content of sample extracts of *A. absinthium* and *A. calamus*. (%)

**4. CONCLUSION**

The comparative phytochemical and antioxidant profiling of *A. absinthium* and *A. calamus* highlights a pronounced superiority of *A. absinthium* in terms of its total polyphenolic content, flavonoid concentration, and DPPH free radical scavenging activity. Specifically, *A. absinthium*  exhibited significantly higher levels of polyphenols (128.4 mg GAE/g) and flavonoids (18 mg QE/g), along with potent antioxidant capacity (63.40%), compared to *A. calamus*, which recorded 12.02 mg GAE/g polyphenols, 5.65 mg QE/g flavonoids, and 38.7% DPPH activity. These biochemical markers are well-established indicators of antioxidant potential, suggesting that *A. absinthium* holds greater promise as a source of natural antioxidants.

The results are consistent with previous studies that have documented the rich bioactive profile of *A. absinthium* species, including their anti-inflammatory, antimicrobial, and anticancer activities (Wang et al., 2020; Tayarani-Najaran et al., 2021). In contrast, although *A. calamus* has traditional medicinal relevance, particularly in Ayurvedic and traditional Chinese medicine, its comparatively lower antioxidant metrics may limit its efficacy in modern therapeutic or nutraceutical applications unless used in combination with other potent agents (Ahmed et al., 2022). In light of growing global interest in plant-based antioxidants for disease prevention and health promotion, *A. absinthium* may serve as a superior candidate for further development in pharmaceutical, cosmetic, and functional food industries. Future research should focus on isolating specific bioactive compounds from *A. absinthium*, conducting in vivo studies, and exploring formulation-based applications for targeted therapeutic benefits.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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