In-vitro Antioxidant Activity and Preliminary Phytochemical Analysis of different leaf extracts of *Hemionitis arifolia*

Abstract

Objective

To evaluate the preliminary phytochemical content and antioxidant potential of the hydroalcoholic leaf extracts of *Hemionitis arifolia*.

Methods

Total phenolic, flavonoid and alkaloid contents were evaluated using spectrophotometric methods. The free radical scavenging activity of the leaf hydroalcoholic extract were evaluated against $DPPH^+$, $ABTS^+$, Reducing power assay and nitric oxide assay were determined.

Results

The hydroalcoholic concentrate of *H. arifolia* uncovered the most elevated polyphenol content when contrasted and the other phytoconstituents. Absolute phenol content of the hydroalcoholic separate was observed to be 31.78%, flavonoid content is 1.02% and Alkaloid content is 30.40% individually. The Solvent concentrates showed huge cell reinforcement movement, with hydroalcoholic extract. ABTS Assay, DPPH assay, Reducing power assay and Nitric oxide assay where the Inhibition concentration were 667.75µg/ml, 734.25 µg/ml, 791.58 µg/ml and 899.67 µg/ml.

Conclusion

This study suggests that hydroalcoholic leaf extracts of *H. arifolia* could be a potential source of natural antioxidant and justifies its use in ethno-medicine.

Keywords: *H.arifolia*, Qualitative phytochemical activity, Quantitative Phytochemical activity, free radicals, polyphenol, Antioxidant activity.

Introduction

The search for new cures has always been guided by traditional information on medicinal plants. Despite the introduction of modern high-throughput drug disclosure and

screening technologies, traditional information frameworks have provided suggestions to the disclosure of critical drugs [1]. Traditional medicinal herbs are frequently less expensive, more easily available, and more effectively consumable, whether in its raw form or as basic restorative arrangements. Traditional therapeutic approaches now make up a significant portion of reciprocal or elective prescriptions. Because of their dynamic chemical ingredients, these simple restorative arrangements frequently intercede therapeutic reactions, despite the fact that their adequacy and system of activity have not been attempted scientific method much of the time [2].

Plants have been used as a source of food and medicine for thousands of years. They're not only high-nutrient veggies, but their various sections (leaf, natural product, and root) are also used for health-related applications. The interest of the logical class in the examination of plant starting accumulations is growing all around the world, particularly in agricultural countries where natural medicines are widely used for basic health needs [3]. It is well known that medicinal plants have been used to cure a variety of maladies, including asthma, stomach difficulties, skin diseases, respiratory and urinary complications, and liver and cardiovascular infection, since ancient times [4,5]. This observational data comes from the plant protection framework, which generates various builds with various sub-atomic designs that are far superior to those obtained from engineered items [6]. As a result, the explanation of new dynamic standards has sparked a lot of interest.

Plant items' beneficial effects can be attributed to the natural activities of phytochemical and cell reinforcement ingredients such as phenolic compounds, pro-anthocyanidin, minerals, carotenoids, flavonoids, and Saponin [7,8]. Recently, there has been a surge in interest in conventional cancer prevention drugs, with the goal of employing them to reduce the harmful effects of free radicals in the human body [9]. Due to the proof of vitality and wellness, the free extremist rummaging characteristics of therapeutic plants considered as typical cell reinforcements are exploited in a few clinical applications [10]. Cell reinforcements are synthetic compounds that inhibit oxidation by preventing the formation of free radicals that destroy healthy cells, thereby treating and managing chronic illnesses such as cardiovascular infections, diabetes, obesity, and a variety of disorders [11].

Concentrations on normal mixtures with cancer prevention agent exercises have seen huge development in the last two decades, since a significant amount of proof has shown that cell damage caused by oxidative pressure is a significant factor in the maturing and progression of a wide range of pathologies, including immune system illnesses, irresistible or potentially provocative infections, and degenerative and neoplastic diseases [12,13]. The

importance of the search for natural items with cancer-prevention properties is highlighted in this way, since they can prevent, balance out, or free revolutionaries before they attack organic focuses in cells (DNA, proteins, and lipids) [14]. Plants are frequently used to treat a variety of illnesses without being aware of their deadly potential, which can be harmful to human health. One of the most significant concerns in the use of natural products is the belief that the outcomes of plant development are free of unfavourable reactions and harmful effects [15].

Hemionitis arifolia has a place with the family Pteridaceae. Short erect rhizomes covered in caramel tight scales give rise to *H.arifolia*. There are two types of fronds. The stipes (follows) of fruitful (spore-bearing) fronds are generally longer than those of sterile fronds. The frond's edge (lamina) is 3–6 cm (1.2–2.4 in) long by 2–4 cm (0.8–1.6 in) wide, with a heart-moulded base and a reasonably adjusted pinnacle. It is attached to the stipe at a point. The upper side of the fronds is caramel green, while the underside is brown. Hence, the current study were carried out for phytochemical screening and antioxidant activity of the *H.arifolia* extracts. This study was designed and it will be extended in future to identify the bioactive constituents of the sample which will treat cancer.

Materials and Methods

Collection and Extraction of Plant material

H. arifolia leaves were identified and collected and stored in Alpha Omega Hi-Tech Bio Research Centre, Salem (Voucher No: AORF128). The Leaves are freshly collected and cleaned with running tap water and again rinsed in distilled water and it was shade dried. The dried leaves were grinded and stored. The extraction was carried out in Soxhlet apparatus with 250ml of various solvents (Chloroform, Ethyl acetate, Ethanol, Methanol and Hydroalcohol). The sample was taken with coarse powder of 25g. The extracts were taken and stored in refrigerator for further use.

Figure 1: *H. arifolia* leaves

Phytochemical Screening

Preliminary phytochemical analysis was carried out for *H. arifolia leaf* extracts of as per standard methods described by Brain and Turner and Evans [16,17].

Detection of alkaloids

The extracts were dissolved and filtered separately in diluted hydrochloric acid. The presence of alkaloids was determined using the filtrate. Mayer's reagent was used to handle the filtrates. The presence of alkaloids is demonstrated by the production of a yellow cream precipitate.

Detection of Flavonoids

H_2SO_4 test: A few drops of H_2SO_4 were added to the extracts. The presence of flavonoids is indicated by the orange colour development.

Detection of Steroids

2ml of acetic anhydride was added to 0.5 g of extracts, followed by 2ml of H_2SO_4 for each. The colour shift from violet to blue or green in some samples shows the presence of steroid.

Detection of Terpenoids

Salkowski's test: 2ml chloroform and concentrated H_2SO_4 were carefully added to form a layer with 0.2 g of the total plant sample extract (3ml). A reddish-brown hue of the interior face showed the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: Approximately 0.2 g of the extract was boiled in a water bath with 10% HCl for a few minutes. It's been filtered and given time to cool. $CHCl_3$ was added in equal amounts to the filtrate. A few drops of 10% NH_3 have been added to the mixture and heated. The presence of anthraquinones is shown by the pink colour formation.

Detection of Phenols

Ferric chloride test: Extracts were treated with a few drops of a 5% ferric chloride solution. The presence of phenol is indicated by the formation of a bluish black colour.

Detection of Saponins

Froth test: About 0.2 g of powder was shaken with 5ml of distilled water. Saponin production (appearance of creamy stable consisting of tiny bubbles).

Detection of Tannins

Ferric chloride test: In a water bath, a small amount of extract was combined with water and boiled. The mixture was filtered, and 0.1 percent ferric chloride was added to the filtrate. A dark green colour indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled with 0.2ml each of Fehling solution A and B in a water bath. A red precipitation shows the existence of sugar.

Detection of Oils and Resins

Spot test: The test technique was carried out on filter paper. It creates a transparent appearance on the filter paper. The presence of oils and resins is indicated.

Quantitative Phytochemical Analysis

Determination of Alkaloids

Alkaloid determined by Harborne [18] standard procedure. In a 250 ml beaker, 5g of the hydroalcoholic concentrate of *H. arifolia* test was weighed, and 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 4 hours. This was separated, and the concentrate was concentrated to one-fourth of the first volume on a water shower. Drop by drop, concentrated ammonium hydroxide was added to the concentrate until the precipitation was complete. After allowing the entire arrangement to settle, the encouraged was collected and rinsed with mild ammonium hydroxide before being filtered. The alkaloid, which was dried and gauged, is the build-up.

Determination of Flavonoids

Ten grams of hydroalcoholic extract of *S. alata* test was over and over separated with 100ml of 80% fluid methanol at room temperature. The combination was then sifted through a channel paper into a pre-weighed 250ml measuring utensil. The filtrate was moved into a water shower and permitted to dissipate to dryness and gauged. The quantity of flavonoid was determined by contrast [19].

Determination of Total phenols

The fat free example was taken in beaker with 50 ml of ether for the extraction of the phenolic part for 15 min. 5 ml of the concentrate was pipetted into a 50 ml conical flask, then, at the point 10 ml of refined water was added. 2 ml of ammonium hydroxide arrangement and 5 ml of concentrated amyl liquor were additionally added. The hydroalcoholic extract of *H. arifolia* tests was left to respond for 30 min for shading advancement. This was estimated at 505nm [20].

Antioxidant activity

DPPH Radical Scavenging Activity

Molyneux [21] Method completed DPPH revolutionary searching movement. To 1.0 ml of 100.0 µM DPPH arrangement in methanol, a similar volume of hydroalcoholic leaf extricate in *H. arifolia* test in various centralization of was added and 30 minutes brooded in dim. The change in shading was identified with a spectrophotometer at 514 nm as far as absorbance. 1.0 ml of methanol was added to the control tube rather than the test.

Reducing Power assay

Utilizing the procedure referenced the decrease power of hydroalcoholic leaf remove in *H. arifolia* not really set in stone [22]. In 0.2 M phosphate support pH, 6.6 containing 1% ferrocyanide, a sequential weakening of the concentrate (1000, 750, 500, 250 and 50 µg/mL) was performed. The combination was 20 minutes brooded at 50^{o}C. A piece of this mix (5 mL) was added to 10% trichloroacetic acid (TCA, 2.5 mL) and centrifuged for 10 minutes at 3,000 g. Isolated and mixed the supernatant with refined water (2.5 mL) containing 1% ferric chloride (0.5 mL). This present blend's absorbance was assessed at 700 nm. The absorbance force could be the estimation of the concentrate's cancer prevention agent movement.

ABTS radical scavenging activity

ABTS revolutionary searching movement of the hydroalcoholic leaf extricate in *H. arifolia* not really set in stone as indicated by Re [23]. The ABTS +cation extremist was created by the response between 5 ml of 14 mM ABTS arrangement and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) arrangement, put away in obscurity at room temperature for 16 hrs. Prior to utilize, this arrangement was weakened with ethanol to get an absorbance at 734 nm. The plant removed at different focuses with 1ml of ABTS arrangement was homogenized and its absorbance was recorded at 734 nm. Ethanol spaces were run in each examine, and all estimations were done after something like 6 min. Also, the response combination of standard gathering was acquired by blending 950 µl of ABTS.+ arrangement and 50 µl of BHT. Concerning the antiradical movement, ABTS searching capacity was communicated as IC_{50} (µg/ml).

Nitric oxide scavenging activity

Sodium nitroprusside in fluid arrangement at physiological pH precipitously creates nitric oxide (NO), which cooperates with oxygen to deliver nitrite particles, which can be

assessed utilizing Griess Illosvosy response [24]. Scavengers of NO compete with oxygen, prompting diminished creation of NO and a pink shaded chromophore is shaped. The absorbance of these arrangements was estimated at 540 nm against the comparing clear arrangements.

Inhibition Percentage Calculation

Percentage of inhibition was calculated from the equation

[(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100.

Inhibition concentration 50% value was calculated using Graph pad prism 5.0.

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Results and Discussion

The essential innovation in any phytochemical screening technology is extraction. This includes the extraction of restoratively dynamic pieces of plant tissues using specific solvents using established procedures. The existence of bioactive ingredients known to have substantial pharmacological effects was discovered throughout the current review's phytochemical investigations [25]. The metabolite blends obtained as a result of this procedure from plants are fairly complex metabolite blends in fluid or semi-solid form or dry powder structure, and are intended for pharmacological applications. During extraction, solvents penetrate the dense plant material and solubilize chemicals to the same extent [26].

Qualitative phytochemical analysis

H. arifolia leaves were tested for phytochemicals in a variety of solvents, including chloroform, ethyl alcohol, ethanol, methanol and hydroalcoholic. Alkaloids, Flavonoids, Steroids, Terpenoids, Arthroquinone, Phenols, Saponin, Tannin, and Carbohydrates were found in these concentrates after hydroalcoholic separation. When compared to other concentrations, the hydroalcoholic separation is said to be excellent. No tests have been found in the literature to demonstrate the presence of any of these phytoconstituents, nor at any level, and only coumarins have been reported for the species [6].

Alkaloids are a group of naturally occurring synthetic mixes that are mostly composed of fundamental nitrogen iotas. Their pain-relieving abilities have been established [27]. Plant alkaloids have the potential to be used as sedative specialists in medicine [28]. Alkaloids have important physiological effects once they've been controlled in animals, which explains their widespread application in medicine development [29,30].

Table 1: Qualitative Phytochemical Analysis of *H. arifolia* Leaf Extract

Phytochemicals	Extracts				
	Chloroform	Ethyl acetate	Ethanol	Methanol	Hydroalcohol
Alkaloids Mayer's test Wagner's test	- -	- -	+ +	+ +	++ ++
Flavonoids Lead acetate test H_2SO_4 test	- -	- -	+ +	++ ++	++ ++
Steroids Liebermann-Burchard test	+	-	+	++	++
Terpenoids Salkowski test	-	-	+	++	++
Arthroquinone Borntrager's test	-	-	-	-	+
Phenols Ferric chloride test Lead acetate test	- -	- -	+ +	++ ++	++ ++
Saponin	-	-	-	-	+
Tannin	-	-	+	+	+
Carbohydrates	+	+	+	+	+
Oils & Resins	+	+	-	-	-

Plants provide regular cancer prevention agents in the form of phenolic mixes such as flavonoids, phenolic acids, and so on [31]. Flavonoids and tannins are a large group of compounds that act as cell reinforcements or free radical scavengers. Flavonoids are water-soluble phytochemicals that reduce free radicals by extinguishing, up-directing, or securing cell reinforcement defences, as well as chelating innovative intermediate compounds [32]. Phenolic chemicals are important because they have a high cell reinforcement potential, which protects the human body from oxidative stress, which can cause diseases such as cancer, cardiovascular problems, and ageing [20]. Tannins contribute to standards of qualities such as quick damaged recovery and stimulated mucous film. Steroids were also identified, and their therapeutic value may be determined by their interactions with other substances, such as sex chemicals [33].

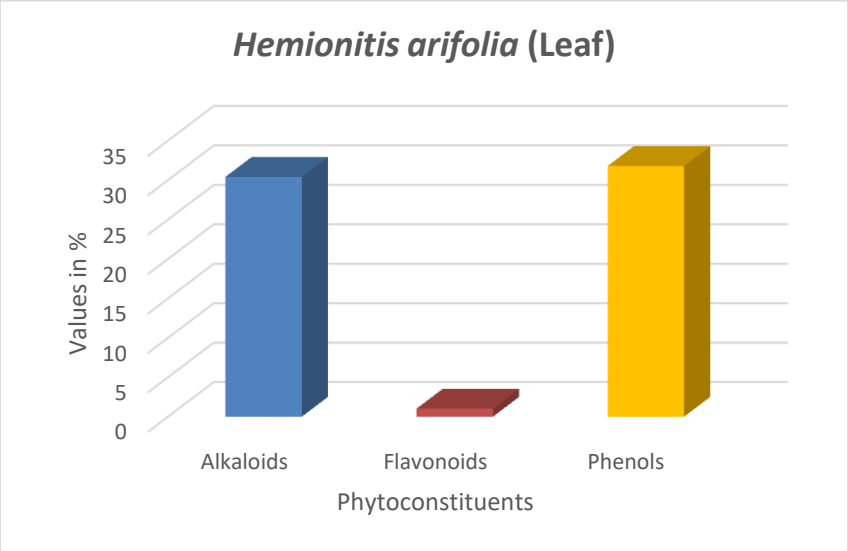
Quantitative Phytochemical Analysis

As shown in Table 2, the goal assessment of *H. arifolia* hydroalcoholic separate was reached. For several phytoconstituents, only the hydroalcoholic separate was tested. The quantitative alkaloid content test yielded 30.40 percent from plant separately. When compared to other phytoconstituents, phenolic content (31.78 percent) shows more variation represented in Figure 2 and Table 2. Alkaloids are highly toxic intensifiers that primarily affect the central sensory system [34].

Table 2: Quantitative Phytochemical Analysis of *H. arifolia* Hydroalcoholic leaf Extract

S.No	Phytoconstituents	% Hydroalcoholic leaf Extract of *H. arifolia*
1	Alkaloids	30.40
2	Flavonoids	1.02
3	Phenols	31.78

Figure 2: Quantitative Phytochemical Analysis of *H. arifolia* Hydroalcoholic leaf Extract



The calculated flavonoid esteems from the examples were 1.02 percent. The sickness can be cured with alkaloid, flavonoid and phenols. Hydroalcoholic removes were shown to have a high material content when compared to other concentrates used for medicinal

purposes. As a result, the findings of this study suggest that identified phytochemical combinations may be the bioactive ingredients responsible for the efficacy of *H. arifolia* concentrated on plants. It's also been confirmed that the presence of antibacterial, cancer-prevention, and anticancer capabilities in several of these mixes. In this way, the investigation may conclude that leaf concentrates could be a valuable source of valuable in the treatment of some microbial contaminants for the mechanical synthesis of pharmaceuticals. The existence of these phytochemicals can be attributed to the bioactive characteristics of most therapeutic plants, including the plant in question, which is responsible for ethno-pharmacological investigations.

In-vitro Antioxidant Activity

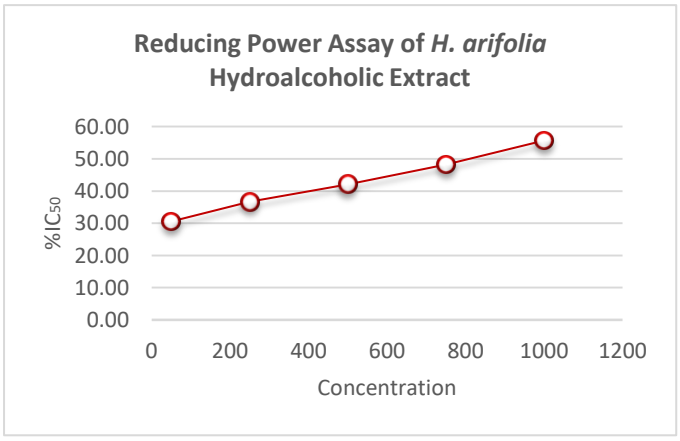
Free radicals are believed to have a prominent role in a wide range of neurotic symptoms. Cancer preventive agents combat free radicals and protect us from many illnesses. They either investigate the responsive oxygen species or secure the cancer prevention agent guard equipment as part of their work [35]. Antioxidant action was performed for a variety of assays, including the reducing power test, DPPH test, ABTS test, and Nitric oxide test represented in Table 3 and Figure 3.

Table 3: *In-vitro* antioxidant activity of *H. arifolia* Hydroalcoholic leaf Extract

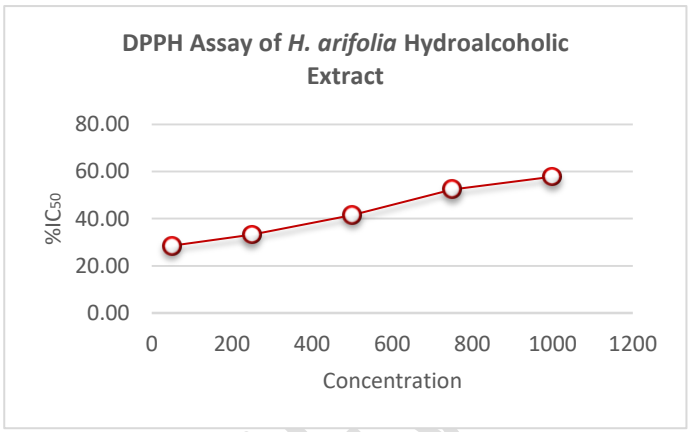
S.No	Conc	Reducing Power Assay % Inhibition conc	Inhibition concentration	DPPH Assay % Inhibition conc	Inhibition concentration	ABTS Assay % Inhibition conc	Inhibition concentration	Nitric Oxide Assay % Inhibition conc	Inhibition concentration
1	50	30.61	791.58	28.57	734.25	32.65	667.75	23.81	899.67
2	250	36.73		33.33		41.50		29.93	
3	500	42.18		41.50		47.62		37.41	
4	750	48.30		52.38		51.70		45.58	
5	1000	55.78		57.82		57.14		53.06	

Figure 3: *In-vitro* antioxidant activity of *H. arifolia* Hydroalcoholic leaf Extract

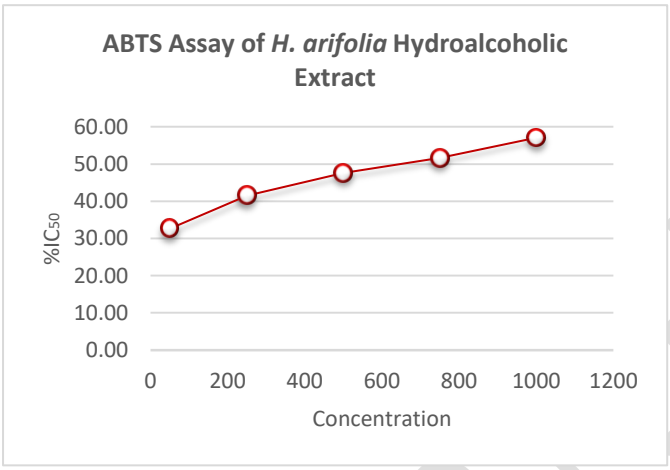
Reducing Power Assay



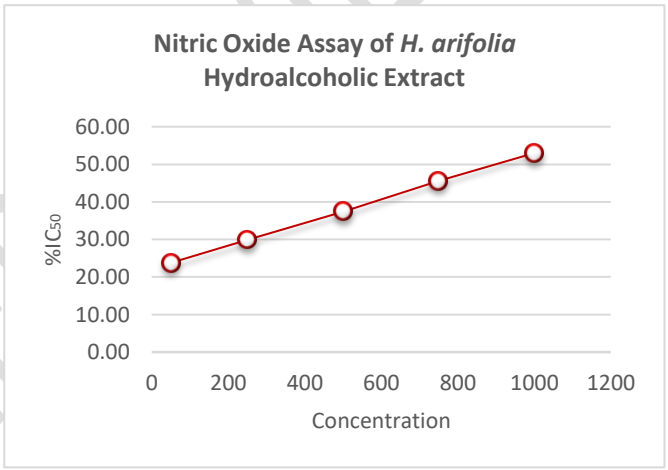
DPPH Assay



ABTS Assay



Nitric Oxide Assay



For the unique measure 50, 250, 500, 750, and 1000 g/ml, different concentration was done. In a declining power test, the yellow shade of the test arrangement shifts to green as the test example's strength decreases. The Fe3+/ferricyanide complex is reduced to the ferrous structure when the reductants are present in the arrangement. As a result, Fe2+ can be detected by measuring absorbance at 700 nm. The restraint focus half was found to be 791.58 g/ml in a decreasing power test. According to previous reports, the decreasing properties have been shown to be used to apply cell reinforcement activity by supplying a hydrogen molecule to break the free extreme chain [36]. At 700 nm, expanding absorbance indicates a decrease in capacity. The 2, 2′-diphenyl-1-picrylhydrazyl revolutionary (DPPH) purple-shaded arrangement fading can be used to measure the electron donating capacity of typical goods [37]. The method is based on finding DPPH by expanding an extreme animal category or using cell reinforcement to decolorize the DPPH layout. The degree of shading variation is determined by the cell reinforcements' fixation and strength. The response blend's absorbance

has dropped dramatically, indicating the compound's important free extremist seeking action [38]. In the DPPH test, Rate Inhibition 50 was found to be 734.25 g/ml, 667.75 g/ml in the ABTS extremist detection test, and 899.67 g/ml in the Nitric Oxide Assay. The ABTS extremist rummaging test uses a technique that uses ABTS and potassium persulfate to create a blue/green ABTS+ chromophore. The ABTS extremist cation is formed when ABTS is oxidised with potassium persulfate, and its reduction is measured spectrophotometrically at 745 nm in the presence of hydrogen-giving cancer prevention drugs. Every one of the divisions exhibited a strong ABTS rummaging action, according to various scientists [39].

Conclusion

Several research supporting the therapeutic benefits of plants have confirmed the presence of these phytochemicals. As a result, extracts from this plant could be an excellent source of valuable medications. Preliminary qualitative testing is helpful in detecting bioactive principles and could lead to drug discovery and development. Phytochemical studies have demonstrated that the hydroalcoholic extract of *H. arifolia* has a large number of potent phytoconstituents that produce physiological responses. Nonetheless, a careful investigation of plant material is required in order to discover this plant's secret therapeutic potential. It's also crucial to use a variety of phytochemical procedures to isolate, purify, and classify the active ingredients found in this plant, which could a day be used to make medications.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use one in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the sample because we do not intend to use these sample as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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