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 alkaliods 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 extricate showing most noteworthy cancer prevention agent capacity in connection with the polyphenol substance. In light of the IC_{50} esteems, hydroalcoholic concentrate of the leaf uncovered the best extremist searching action in ABTS Assay, DPPH assay, Reducing power assay and Nitric oxide assay.

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.

Comment [D1]: Kindly check the spelling

Comment [D2]: The botanical name of plant should be in italics and space between the species and genus. Make this correction throughout the manuscript

Comment [D3]: Kindly write only the results of this work.

Comment [D4]: Suggestion: change this by considering

Introduction

Traditional information on therapeutic plants has consistently directed the quest for new fixes. Disregarding the coming of present day high throughput drug disclosure and screening strategies, conventional information frameworks have offered hints to the revelation of important medications (Buenz *et al*, 2004). Customary therapeutic plants are regularly less expensive, locally accessible and effectively consumable, crude or as basic restorative arrangements. These days, customary therapeutic practices structure a fundamental piece of reciprocal or elective prescriptions. Despite the fact that their adequacy and system of activity have not been tried deductively much of the time, these straightforward restorative arrangements regularly intercede helpful reactions because of their dynamic compound constituents (Park and Pezzutto, 2002).

Plants have for quite some time been utilized as a wellspring of food and medication. They not just fill in as vegetables of high nutritive worth, yet their various parts (leaf, natural product, and root) are utilized for wellbeing medicinal purposes. The interest of the logical class in the investigation of accumulates of plant beginning is expanding around the world, particularly in agricultural nations where the utilization of natural meds is broadly utilized for their fundamental wellbeing needs (Yadav, 2018). It is realized that therapeutic plants have been utilized worldwide since old occasions for the treatment of different illnesses, including asthma, stomach problems, skin sicknesses, respiratory and urinary complexities, and liver and cardiovascular infection (Tian *et al*., 2014; Egamberdieva *et al*., 2016). This observational information comes from the plant protection framework, which creates various builds with assorted sub-atomic designs, far better than those got from engineered items (Pradeepa *et al*., 2014), so the extraordinary interest in the explanation of new dynamic standards.

The advantageous impacts of plant items can be credited to the natural exercises of their phytochemical and cell reinforcement constituents, for example, phenolic compounds, pro-anthocyanidin, nutrients, carotenoids, flavonoids, and Saponin (Dinda *et al*., 2007; Francis *et al.*, 2002). As of late, there has been an expanding interest in regular cancer prevention agents, fully intent on using them to diminish the poisonous impacts of free extremists in the human body (Zaouali *et al*., 2010). The free extremist rummaging properties of therapeutic plants considered as regular cell reinforcements are used in a few clinical applications due to the confirmation of viability and wellbeing (Al-Snafi, 2016). Cell reinforcements are synthetic substances that the repress oxidation measure by forestalling the arrangement of free revolutionaries that cause harm to sound cells, along these lines treating

and overseeing persistent sicknesses like cardiovascular infections, diabetes, weight, and a few types of diseases (Lillioja *et al.*, 2013).

Just over the most recent twenty years, concentrates on zeroed in on normal mixtures with cancer prevention agent exercises have shown huge development, since a significant measure of proof has demonstrated that cell harm brought about by oxidative pressure has been viewed as a significant factor in maturing and in the improvement of a wide assortment of pathologies, like immune system illnesses, irresistible or potentially provocative infections, and degenerative and neurodegenerative sicknesses (Ayres *et al.*, 2009; Ulewicz-Magulska and Wesolowski, 2019). In this way, the significance of the quest for normal items with cancer prevention agent impact is underlined, as they can forestall, balance out, or handicap free revolutionaries before they assault organic focuses in cells (DNA, proteins, and lipids) (Vasconcelos *et al.*, 2007). Frequently, individuals use plants to treat an assortment of sicknesses, without knowing their poisonous potential, which can be destructive to human wellbeing. One of the primary issues in the utilization of regular items is the conviction that results of plant beginning are liberated from unfavourable responses and poisonous impacts (Clarke *et al.*, 2007).

H.arifolia has a place with the family Pteridaceae. *H.arifolia* develops from short erect rhizomes covered with caramel tight scales. The fronds are of two kinds. Fruitful (spore-bearing) fronds have stipes (follows) that are normally any longer than those of sterile fronds. The edge (lamina) of the frond is normally 3–6 cm (1.2–2.4 in) long by around 2–4 cm (0.8–1.6 in) wide, with a heart-moulded base and a fairly adjusted pinnacle. It is held at a point to the stipe. Fronds are caramel green on the upper side, brown on the lower side. Hence, in the current concentrate in-vitro cell reinforcement action and primer phytochemical examination was completed in *H.arifolia* separates, to characterize the danger related with phyto-therapy, just as guide research for the confinement of specific mixtures until the advancement of new medications.

Comment [D5]: Kindly provide the full name here

Comment [D6]: Kindly provide the information of the medicinal properties with proper citations about this plant and mention if there is any study related to antioxidant and phytochemical analysis of various parts of this plant with all the details of parts used solvents used for extraction. Kindly explain that why this study has been planned.

Materials and Methods

Collection and Extraction of Plant material

H.arifolia leaves were assembled and the example was put away in the Alpha Omega Hi-Tech Bio research focus. With running regular water, the plant materials were cleaned and dried shade. The leaves have been squashed to processor generally powdered. In 250ml of every dissolvable (Chloroform, ethyl acetic acid derivation, ethanol, methanol and hydro-liquor), these coarse powders (25 g) were then exposed to continuous extraction utilizing

Comment [D7]: What is the meaning of this line? It does not make any sense.

Soxhlet mechanical assembly. The gathered concentrates were saved then taken up for additional examinations. The DMSO (Dimethyl sufloxide) is go about as broken up solvents for these concentrates.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for *H.arifolia leaf* extracts of as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Extracts were separately dissolved and filtered in diluted hydrochloric acid. The filtrate was used to assess alkaloids existence. Filtrates have been handled with the reagent of Mayer. The formation of a precipitate of yellow cream shows the existence of alkaloids.

Detection of Flavonoids

H_2SO_4 test: Extracts with few drops of H_2SO_4 have been handled. Orange colour formation shows the existence of flavonoids.

Detection of Steroids

0.5 g of the extracts were added with 2ml of acetic anhydride, each with 2ml of H_2SO_4. In some samples, the colour shifted from violet to blue or green indicates steroid existence.

Detection of Terpenoids

Salkowski's test: 0.2 g of the entire plant sample extract was carefully added to form a layer with 2ml of chloroform and concentrated H_2SO_4 (3ml). The presence of terpenoids was indicated by a reddish-brown coloration of the internal face.

Detection of Anthroquinones

Borntrager's test: Approximately 0.2 g of the extract was boiled for a few minutes in a water bath with 10 percent HCl. It has been filtered and permitted to cool down. The filtrate has been added equal quantity of $CHCl_3$. Few 10 percent NH_3 drops have been added and heated to the blend. Pink colour formation shows the existence of anthraquinones.

Detection of Phenols

Ferric chloride test: With few drops of 5 percent ferric chloride solution, extracts were handled. Bluish black colour formation shows phenol existence.

Detection of Saponins

Froth test: With 5ml of distilled water, about 0.2 g of the powder was shaken. Formation of saponins (appearance of creamy stable consisting of tiny bubbles).

Detection of Tannins

Ferric chloride test: A small amount of extract was mixed with water and heated in a bath of water. The blend was filtered and added to the filtrate 0.1 percent ferric chloride. The presence of tannins is indicated by a dark green colour.

Detection of Carbohydrates

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

Detection of Oils and Resins

Spot test: On filter paper, the test method was implemented. It creates on the filter paper a transparent appearance. The presence of oils and resins is indicated.

Quantitative Phytochemical Analysis

Determination of Alkaloids

Alkaloid determined by Harborne (1973) strategy. 5g of the hydroalcoholic concentrate of *H.arifolia* test was weighed into a 250 ml beaker and 200 ml of 10% acidic corrosive in ethanol was added and covered and permitted to represent 4 hrs. This was separated and the concentrate was focused on a water shower to one fourth of the first volume. Concentrated ammonium hydroxide was added drop astute to the concentrate until the precipitation was finished. The entire arrangement was permitted to settle and the encouraged was gathered and washed with weaken ammonium hydroxide and afterward sifted. The build-up is the alkaloid, which was dried and gauged.

Determination of Flavonoids

Ten grams of hydroalcoholic concentrate of *H.arifolia* 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 utencil. The filtrate was moved into a water shower and permitted to dissipate to dryness and gauged. The rate flavonoid was determined by contrast (Krishnaiah *et al*, 2009).

Determination of Total phenols

The fat free example was overflowed 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 flagon, then, at that 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 concentrate of

H.arifolia tests were left up to imprint and left to respond for 30 min for shading advancement. This was estimated at 505nm (Siddhuraju and Decker, 2003).

Antioxidant activity

DPPH Radical Scavenging Activity

Molyneux Method (2004) 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 (Yen *et al*., 1994). In 0.2 M phosphate support pH, 6.6 containing 1% ferrocyanate, 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 corrosive (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 *et al*., 1999. 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 of 0.700 ± 0.020 at 734 nm. The plant remove 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 (Garrat, 1964). Foragers of NO contend 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.

IC_{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

In any phytochemical screening methodology, the fundamental advance is extraction. This includes the extraction by standard methods of restoratively dynamic bits of plant tissues utilizing specific solvents. Phytochemical investigations of the current review uncovered the presence of bioactive constituents known to have significant pharmacological impacts (Musa *et al*., 2008). The items consequently got from plants are moderately perplexing metabolite blends, in fluid or semi-strong state or in dry powder structure, and are planned for pharmacological employments. Solvents enter into the strong plant material during extraction and solubilize compounds with equivalent extremity (Ncube *et al*., 2008).

Qualitative phytochemical analysis

Phytochemical evaluating for *H.arifolia* leaves were done for various solvents specifically Chloroform, ethyl acetic acid derivation, ethanol, methanol and hydro-liquor. In these concentrates hydroalcoholic separate uncovered the presence of Alkaloids, Flavonoids, Steroids, Terpenoids, Arthroquinone, Phenols, Saponin, Tannin and Carbohydrates. The hydroalcoholic separate is by all accounts great when contrasted with different concentrates. No examinations have been seen in the writing to demonstrate the presence of every one of these phytoconstituents, neither at sort, and just coumarins was portrayed by (Pradeepa *et al.*, 2014) for the species.

Table 1: Qualitative Phytochemical Analysis of *Hemionitis 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	+	+	-	-	-

Alkaloids are a class of normally happening synthetic mixtures that contain basically fundamental nitrogen iotas. Their pain relieving properties have been recognized (Ndhlala *et al.,* 2010). The alkaloids in plants might be utilized as sedative specialists in medication (Newman *et al*., 2003). Once controlled to creatures, alkaloids have critical physiological impacts and thus their wide use in drug improvement medication (Okwu *et al*., 2006; 2004). Regular cancer prevention agents come principally from plants as phenolic mixtures like flavonoids, phenolic acids, and so on, (Okwu, 2005). Flavonoids and tannins are a huge gathering of mixtures which go about as essential cell reinforcements or free extreme scroungers. Flavonoids are water solvent phytochemicals which, by extinguishing, up-directing or securing cell reinforcement protections and chelating revolutionary middle of the road compounds, lessen free extremists ((Pandey and Debnath, 2011). Phenolic compounds are vital on the grounds that they have a high cell reinforcement potential which shields the human body from oxidative pressure, which can prompt illnesses like disease, cardiovascular issues and maturing (Siddhuraju and Becker, 2003). Tannins add to the astringency properties, for example quicker twisted recuperating and aroused mucous film. Steroids were likewise recognized, and their therapeutic worth might rely upon their relationship to mixtures, for example, sex chemicals (Trease and Evans, 2002).

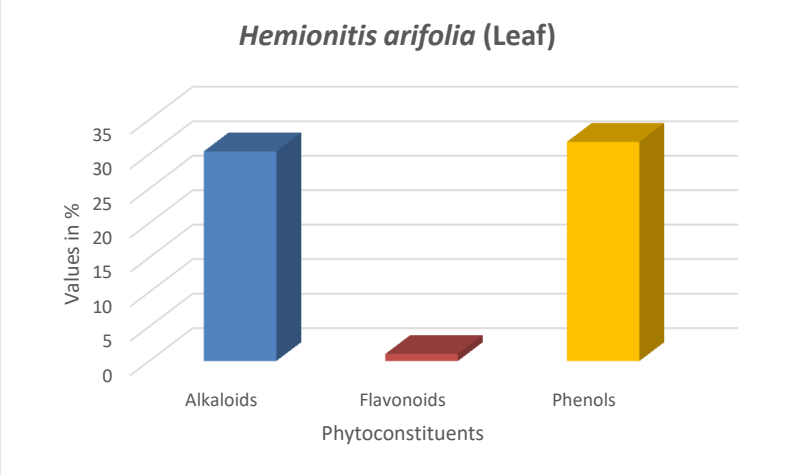
Quantitative Phytochemical Analysis

The target appraisal of *H.arifolia* hydroalcoholic separate covered, as depicted in Table 2. Just hydroalcoholic separate has been checked for various phytoconstituents. The discoveries of quantitative alkaloid content examination got from both the plant separate were 30.40% individually. Phenolic content (31.78%) shows higher movement when contrasted with other phytoconstituents. Alkaloids are intensifies that are pretty much harmful that generally work on the focal sensory system (Vellingiri *et al*., 2011). The flavonoid esteems estimated from the examples was 1.02%. Alkaloid, flavonoid, and phenols can fix the illness. Contrasted and different concentrates utilized for therapeutic purposes, the substance of hydroalcoholic removes was observed to be high. Thusly, the outcomes acquired in this review recommend that the recognized phytochemical mixtures might be the bioactive constituents answerable for the adequacy of the concentrated on plants *H.arifolia*.

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

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

Figure 1: Quantitative Phytochemical Analysis of *H.arifolia* Hydroalcoholic leaf Extract



It has additionally been affirmed that the presence of a portion of these mixtures has antimicrobial, cancer prevention agent and anticancer properties. It could in this manner be closed from the examination that the leaf concentrates could be a wellspring of valuable in the chemotherapy of some microbial contamination for the mechanical production of medications. The presence of these phytochemicals could be credited to the bioactive standards of most therapeutic plants, including the plant under concentrate on that are answerable for ethno-pharmalological exercises.

***In-vitro* Antioxidant Activity**

Free extremists are known to assume a clear part in a wide assortment of neurotic appearances. Cancer prevention agents battle against free extremists and shield us from different infections. They apply their activity either by rummaging the receptive oxygen species or securing the cancer prevention agent guard instruments (Umamaheswari and Chatterjee, 2008). Distinctive Antioxidant action were completed for various tests, for example, lessening power measure, DPPH test, ABTS examine and Nitric oxide test. Different focus were done for the distinctive measure 50, 250, 500, 750 and 1000 µg/ml. In lessening power examine, the yellow shade of the test arrangement changes to green contingent upon the decreasing force of the test example. The presence of the reductants in the arrangement causes the decrease of the Fe3+/ferricyanide complex to the ferrous structure. Thusly, Fe2+ can be observed by absorbance estimation at 700 nm. In diminishing force examine the restraint focus half was observed to be 791.58 µg/ml. Past reports proposed that the lessening properties have been displayed to apply cell reinforcement activity by giving of a hydrogen molecule to break the free extreme chain (Sahreen *et al*., 2010). Expanding absorbance at 700 nm shows an expansion in decreasing capacity.

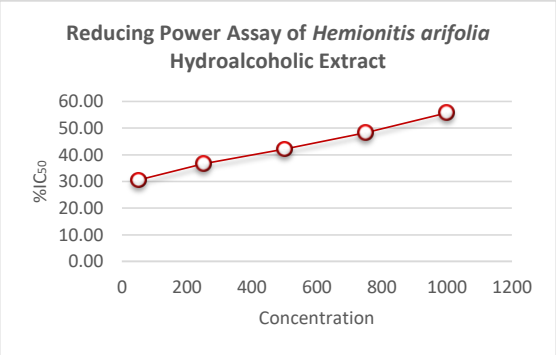
Table 3: *In-vitro* antioxidant activity of *H.arifolia* Hydroalcoholic leaf Extract

S.No	Conc	Reducing Power Assay $\%IC_{50}$	IC_{50}	DPPH Assay $\%IC_{50}$	IC_{50}	ABTS Assay $\%IC_{50}$	IC_{50}	Nitric Oxide Assay $\%IC_{50}$	IC_{50}
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	

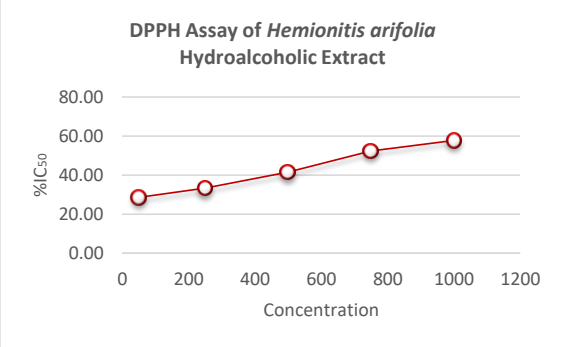
Comment [D8]: There is no term like %IC50. Kindly correct it to %Inhibition throughout the manuscript.

Figure 2: *In-vitro* antioxidant activity of *H.arifolia* Hydroalcoholic leaf Extract

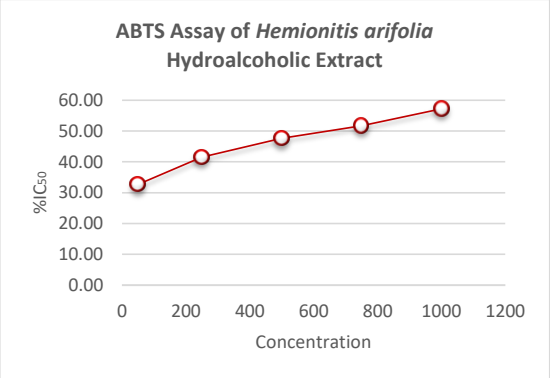
Reducing Power Assay



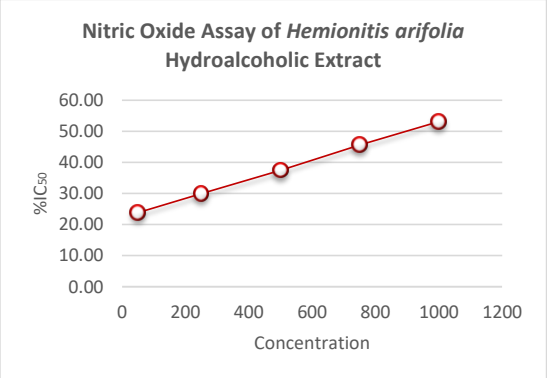
DPPH Assay



ABTS Assay



Nitric Oxide Assay



The electron donation capacity of normal items can be estimated by 2, 2′-diphenyl-1-picrylhydrazyl revolutionary (DPPH) purple-shaded arrangement dying (Nunes *et al*., 2012). The technique depends on searching of DPPH through the expansion of an extreme animal

categories or cell reinforcement that decolourizes the DPPH arrangement. The level of shading change is relative to the fixation and strength of the cell reinforcements. An enormous decline in the absorbance of the response blend demonstrates critical free extremist searching action of the compound under test (Krishnaiah *et al.*, 2011). Rate Inhibition 50 was

observed to be 734.25 µg/ml in DPPH measure, 667.75 µg/ml in ABTS extremist searching test and 899.67 µg/ml in Nitric Oxide Assay.

ABTS extremist rummaging test includes a strategy that produces a blue/green ABTS+ chromophore through the response of ABTS and potassium persulfate. The ABTS extremist cation is created by the oxidation of ABTS with potassium persulfate, its decrease within the sight of hydrogen-giving cancer prevention agents is estimated spectrophotometrically at 745 nm. Every one of the divisions had solid ABTS rummaging action a perception that is upheld by different scientists (Sahreen *et al*., 2011).

Conclusion

The presence of these phytochemicals has been confirmed by several studies contributing medicinal properties to the plants. Extracts from this plant may therefore be viewed as a good source for useful drugs. Preliminary qualitative testing is useful in the detection of bioactive principles, and may result in the discovery and development of drugs. Phytochemical studies have revealed that the hydroalcoholic extract of *H.arifolia* is rich in many active phytoconstituents that give physiological reaction. Nevertheless, to explore this plant's secret therapeutic efficacy, a detailed analysis of plant material is needed in-order. It is also important to follow several phytochemical methods to isolate, purify and classify the active constituents present in this plant, which could later become promising for the production of drugs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products 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.

References

- Al-Snafi AE. Medicinal plants with antioxidant and free radical scavenging effects (part 2): plant based review. IOSR J Pharm. 2016;6:62–82.
- Ayres M. C. C., M. H. Chaves, D. Rinaldo, W. Vilegas, and G. M. Vieira Júnior, "Constituintes químicos e atividade antioxidante de extratos das folhas de *Terminalia fagifolia* Mart. et Zucc," *Química Nova*, vol. 32, no. 6, pp. 1509–1512, 2009.
- Brain KR. and Tuner TD, 1975, The practical evaluation of phytopharmaceuticals. Wright Scientectica Publishers, Bristol. Pp 57-58
- Buenz, I.F.F; Schenepple, D.J; and Motley, T.J. (2004) Technique: bio prospecting historical herbal texts by hunting for new leads in old tomes. Trends in pharmacological Sciences 25, 494-498
- Clarke J. H. R., S. M. K. Rates, and R. Bridi, "Um alerta sobre o uso de produtos de origem vegetal na gravidez," *Infarma*, vol. 19, no. 1-2, pp. 41–48, 2007.
- Dinda B, Debnath S, Harigaya Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chem Pharma Bull (Tokyo). 2007;55:689–728.
- Egamberdieva D., N. Mamedov, E. Ovidi, A. Tiezzi, and L. Craker, "Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: a review," *Journal of Medicinally Active Plants*, vol. 5, no. 2, pp. 59–75, 2016.
- Evans, W.C., Trease and Evans, 1996. Pharmacognosy. 14th Ed. London: WB Saunders Ltd; 119–159.
- Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. Br J Nutr. 2002;88:587–605.
- Garrat DC. The Quantitative Analysis of Drugs, Chapman and Hall Ltd, Japan; 1964; Vol 3; 456-458.
- Harborne JB, (1973). Phytochemical Methods, Chapman and Hall, Ltd., London, pp. 49-188.
- Krishnaiah D, Devi T, Bono A, Sarbatly R (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants Res., 3(2): 67-72.
- Krishnaiah D, Sarbatly R, Nithyanandam RR: A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011, 89: 217-233. Sahreen S, Khan MR, Khan RA: Phenolic compounds and antioxidant activities of Rumex hastatus D. Don. Leaves. J Med Plants Res. 2011, 5: 2755-2765.

- Lillioja S, Neal AL, Tapsell L, Jacobs DR., Jr Whole grains, type 2 diabetes, coronary heart disease, and hypertension: links to the aleurone preferred over indigestible fiber. Biofactors. 2013;39:242–258.
- Molyneux, P. (2004). The use of the stable free radical diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity. Songlanakarin Journal of Science and Technology, 26, 211-219.
- Musa MA, Cooperwood JS, Khan MOF (2008) A Review of Coumarin Derivatives in Pharmacotherapy of Breast Cancer. Current Medicinal Chemistry 15: 2664-2679.
- Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806.
- Ndhlala AR, Finnie JF, Van Staden J: In vitro antioxidant properties, HIV-1 reverse transcriptase and acetylcholinesterase inhibitory effects of traditional herbal preparations sold in South Africa. Molecules 2010; 15:6888-6904.
- Newman DJ, Cragg GM, Snadder KM. Natural Products as sources of new drugs over the period 1981-2002. J. Nat. Prod., 2003; 66(7): 1022-1037.
- Nunes PX, Silva SF, Guedes RJ, Almeida S: Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. 2012
- Okwu DE, Josiah, C.Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006; 5 (4): 357-361.
- Okwu DE, Okwu ME: Chemical composition of Spondias mombin linn plant parts. J Sustain Agric Environ 2004; 6:140-147.
- Okwu DE. Phytochemicals, Vitamins and Mineral contents of two Nigerian Medicinal Plants. Int J Mol Med Adv Sci 2005; 1(4): 375-381
- Pandey M, Debnath M. Phytomedicine: an ancient approach turning into future potential source of therapeutics. J Pharmacognosy Phytother 2011; 3(1):113-17.
- Park, E.J. and Pezzutto, J.M. (2002): Botanicals in Cancer Chemo preventives. Cancer and Metastasis Reviews, 21, 231-255.
- Pradeepa S., S. Subramanian, and V. Kaviyarasan, "Evaluation of antimicorbial activity of *Pithecellobium Dulce* pod pulp extract," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 7, pp. 32–37, 2014.

- Pradeepa S., S. Subramanian, and V. Kaviyarasan, "Evaluation of antimicorbial activity of *Pithecellobium Dulce* pod pulp extract," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 7, pp. 32–37, 2014.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Riceevans C. Antioxidant activity applying an improved ABTS radical decolorization assay. Free Radical Biology and Medicine 1999; 26, 1231–1237.
- Sahreen S, Khan MR, Khan RA: Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food Chem. 2010, 122: 1205-1211.
- Siddhuraju, P. and Decker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (Moringa oleifera Lam) leaves. Journal of Agriculture and Food Chemistry, 51(8), 2144- 2155.
- Tian X.-R., J.-T. Feng, Z.-Q. Ma et al., "Three new glycosides from the whole plant of *Clematis lasiandra* Maxim and their cytotoxicity," *Phytochemistry Letters*, vol. 10, pp. 168–172, 2014.
- Trease GE and Evans WC. Pharmacognosy. Harcourt Publishers Ltd., London, 2002; 72p.
- Ulewicz-Magulska B. and M. Wesolowski, "Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes," *Plant Foods for Human Nutrition*, vol. 74, no. 1, pp. 61–67, 2019.
- Umamaheswari M, Chatterjee TK: In vitro antioxidant activities of the fractions of Coccinnia grandis L. leaf extract. Afr J Trad Compl Altern Med. 2008, 5: 61-73.
- Vasconcelos S. M. L., M. O. F. Goulart, J. B. F. Moura, V. Manfredini, M. S. Benfato, and L. T. Kubota, "Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano," *Química Nova*, vol. 30, pp. 1323–1338, 2007.
- Vellingiri V, Aruna N, Hans KB. 2011. Antioxidant Potential and Health Relevant Functionality of Traditionally Processed Cassia hirsuta L. Seeds: An Indian Underutilized Food Legume.
- Yadav V. K., "Phytochemical and pharmacognostical studies of *Blumea lacera*," *International Journal of Green Pharmacy*, vol. 12, no. 1, 2018.
- Zaouali Y, Bouzaine T, Boussaid M. Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol. 2010; 48: 3144–3152.