

Research Article

Preliminary phytochemical screening, physicochemical analysis and *in-vitro* antioxidant activity of selected *Holigarna* species- Endemic plant species of Western GhatsVinayak Lokapur¹, Varsha Jayakar¹, Manjula Shantaram^{*1, 2}¹Research Scholar, ^{1*}Professor, Department of Studies and Research in Biochemistry, Mangalore University
Jnana Kaveri PG Centre, Chikka Aluvara, Kodagu, Karnataka, India, 571 232^{2*}Director I/c Student Welfare Mangalore University, Mangalagangothri, Dakshina Kannada, Karnataka, India. 574 199

(Received: November 2020

Revised: December 2020

Accepted: December 2020)

Corresponding author: Manjula Shantaram. Email: manjula59@gmail.com

ABSTRACT

Introduction and Aim: Plants from Anacardiaceae family are used in indigenous system of medicine for their anti-arthritic, antibacterial and antioxidant properties. Among them, *Holigarna* species are widely known as toxic members of Anacardiaceae family which are endemic to Western Ghats. It is commonly known as allergic plant which causes contact dermatitis if contacted. However, young leaves and seeds are used against haemorrhoids, obesity, and cancer and skin diseases. Despite the claims and the use of *Holigarna ferruginea*, *Holigarna nigra* and *Holigarna grahamii* for the management of skin diseases in traditional medicine, little is known and documented so the aim of the study was to explore the *in vitro* antioxidant activity and to analyse qualitative and quantitative of phytochemicals along with their physicochemical properties.

Materials and Methods: Physicochemical parameters such as moisture, pH, total ash, acid-insoluble ash, and elemental values were determined. Qualitative and quantitative analysis were carried out along with different antioxidant assays for evaluating the antioxidant activity of aqueous crude extract of *H. ferruginea*, *H. nigra* and *H. grahamii*.

Results: Physicochemical analysis revealed the presence of trace elements. Preliminary phytochemical analysis revealed the presence of tannins, alkaloids, flavonoids, phenols, saponins and steroids in aqueous crude extract of *H. ferruginea*, *H. nigra* and *H. grahamii*. The free radical scavenging activity of all plants exhibited better antioxidant properties.

Conclusion: The results indicated that the leaves contain an appreciable number of bioactive compounds and presence of these phytochemicals, especially the phenols and flavonoids could be the reason behind the use of both plants in ethno-medicine for the management of various ailments.

Keywords: Phytochemical studies; physicochemical parameters; *H. ferruginea*; antioxidant activity; DPPH; endemic plants; *H. nigra*; *H. grahamii*

INTRODUCTION

Traditional herbal medicines were the source for people to heal themselves and plants have been mined for novel secondary metabolites since 1800's. Almost all developing countries throughout the World consider traditional systems of medicine as important health sources (1). Since early 2020, the concern about the front-liners who are fighting against the coronavirus disease 2019 (COVID-19) is worrying as this disease was rising in an alarming rate. Medicinal plants gave a huge contribution in this pandemic. The medicinal plant has regained its attention although it has the perception of being hay or woods (2). Plant based natural products are important for pharmaceutical research as well as for drug development as a source of therapeutic agents. About, 80% of the terrestrial floras are virtually untapped sources of bioactive metabolites (3, 4).

The secondary metabolites present in the leaves, fruits, barks, stems and roots of medicinal plants include phytochemicals like flavonoids and other phenolic compounds, which has led to their therapeutic use in

the treatment of various diseases. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by (5). Phenolic compounds, especially flavonoids, have been investigated for their antioxidant properties and proven to be effective (6). Environmental factors play a crucial role in antioxidant properties of medicinal plants like its variety, climatic and seasonal variations, geographical regions of growth, degree of ripeness, growing practices, and many other factors such as postharvest treatment and processing. In Anacardiaceae, toxic phenols are likely a defence against pests, because they are capable of restricting the growth of pathogenic fungi such as *Alternaria* sp. These compounds play a dual role as both toxic and beneficial to health. At lowest or average quantity, it exerts beneficial effects on cellular response and immune function and at higher levels, they produce oxidative stress that can damage all cells (7). For appropriate determination of antioxidant capacity, the

extraction technique, its conditions, solvent used, and particular assay methodology are important (8).

Plants from Anacardiaceae family are used in indigenous system of medicine for their anti-arthritis, antibacterial and antioxidant properties. Among them, *Holigarna* species are widely known as toxic members of Anacardiaceae family which are endemic to Western Ghats. It is commonly known as allergic plant which causes 'Rhus' – type contact dermatitis (9). The most commonly known facts about this species is that genus *Holigarna* have an acrid juice in all its parts (10) and bark in particular, releases white milky latex which will convert to black colour after exposing to air and that is highly irritating to skin. Its seed is used against haemorrhoids, obesity, and cancer and skin diseases (11). Allergic contact dermatitis due to various members of Anacardiaceae is a common problem among the outdoor workers of the South Kanara and Konkan area. Among these plants *Anacardium occidentale* and *Semecarpus anacardium* have been extensively studied for various purposes. Surprisingly, *Holigarna* species which are widely grown in Kodagu region and Western Ghats are not yet explored. The only species studied to some extent is *H. anottiana* while other species are untouched (12). Retrospective researches revealed that *H. arnottiana* showed the properties like antioxidant, antifeedant, anticancer and also allergic potentials (13).

Despite the claims and the use of *H. ferruginea*, *H. nigra* and *H. grahamii* for the management of skin diseases in traditional medicine, little is known and documented regarding total phenolic content, total flavonoid content, total alkaloid content, total saponin content and antioxidant activity of these medicinally valuable toxic plant species. In the light of this, the present study is intended to explore the *in vitro* antioxidant activity and to analyse qualitative and quantitative of phytochemicals along with their physicochemical properties contained in *H. ferruginea*, *H. grahamii* and *H. nigra*.

MATERIALS AND METHODS

Selection and identification of plants

The plant samples of *H. ferruginea* was collected from Dakshina Kannada district, Karnataka whereas *H. grahamii* and *H. nigra* were collected from Kodagu district, Karnataka, India. The plant materials were identified and authenticated by Dr. H. S. Shenoy, Principal Scientist, Pilikula Nisarga Dhama Herbarium, Mangalore, Karnataka, India vide letter no. SKPND:CR: 113: Herbarium Collection/19-20. The herbarium is kept at PND Herbarium, Mangalore *H. ferruginea* (accession no. 377), *H. grahamii* (accession no. 2366) and *H. nigra* (accession no. 5107) for further reference.

Preparation of plant extracts for physicochemical and elemental analysis

Plant leaves were washed in running tap water to remove the dirt, followed by distilled water, and dried under shade. The dried leaves were grinded into a fine powder using electric blender.

Evaluation of physicochemical characteristics

Physicochemical properties namely pH, moisture, total ash, acid-insoluble ash content, and elemental studies of leaves of *Holigarna ferruginea*, *Holigarna grahamii* and *Holigarna nigra* were determined using standard protocols.

Determination of moisture content

The moisture content of leaf powder of the plants was determined by the weight difference method as mentioned in the protocol of (14). A known quantity of the powdered leaf material (10 g) was spread uniformly in a pre-weighed weighing dish and was heated in an oven at about 105°C. Till the constant weight is formed. The weighing dish was cooled and weighed again. The content of moisture (%) was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Loss of weight}}{\text{Weight of sample}} \times 100$$

Determination of total ash content

A known quantity of the powdered leaf material (1 g) was placed in a pre-weighed silica crucible and was ignited to constant weight by gradually raising the temperature to about 750°C - 760°C until leaf material turns to white colour which indicates the absence of carbon (14). The crucible was cooled and weighed. The content of total ash (%) was calculated using the formula:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of acid-insoluble ash content

To the crucible containing total ash, 25 ml of 2N HCl was added, covered with a watch glass, and gently boiled for 5 minutes. The crucible was cooled and the acid-insoluble content of the ash was collected on ash less filter paper. The filter paper was washed repeatedly with hot water until the filtrate is neutral in reaction. The filter paper with acid insoluble material was taken in a pre-weighed crucible, dried on a hot plate, ignited to a constant weight, cooled, and weighed (14, 15). The acid insoluble ash (%) content was calculated using the formula:

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of pH

A 5 % w/v aqueous solution of leaf materials was prepared and the pH of solutions was determined using a calibrated digital pH meter by following the method described by AOAC (16). The pH meter was

calibrated using pH 4, 7, and 9 standard solutions before determination of pH of the sample.

Determination of the elemental constituent of *H. ferrugenia*, *H. grahamii*, and *H. nigra*

The standard calibration curve method was used to determine the elemental contents (17, 18). 5 g of air-dried sample in an evaporating dish was placed in an oven at 80°C and dried to a constant weight. The sample was placed in a weighing crucible and ignited at 500°C in a hot spot furnace for three hours. The ash material was prepared for the determination of trace element. A portion of 5gm of the ash sample was digested by heating for two min with a mixture of 10 mL each of nitric acid (HNO₃), HCl and a perchloric acid in a 500 mL flask. The aliquot obtained was mixed with 10 mL of 2M HNO₃ and 30 mL of deionised water in a 100 mL volumetric flask. The volume was made up to zero mark with distilled water. Similar procedure was followed for blank sample and standard solution for the various elements. All samples placed in a plastic container and stored in a refrigerator maintained at 4 °C prior to analysis. Flame emission spectrometer (Systronics `Model 128) was used to determine sodium (Na) and potassium (K) concentrations. Other elements, iron (Fe), Chromium (Cr), lead (Pb), zinc (Zn), and were determined by atomic absorption spectrometry with (*GBC Avanta*) at the appropriate wave-length, temperature and lamp current for each element (19).

Preparation of aqueous extracts of *H. ferrugenia*, *H. grahamii*, and *H. nigra* for phytochemical screening and anti-oxidant activity

Plant leaves of *H. ferrugenia*, *H. grahamii*, and *H. nigra* were washed thoroughly in running tap water followed by distilled water to remove the dirt, and dried under shade. The shade dried materials were grinded into a fine powder using electric blender. The 50 grams of powder was mixed with 500ml of water and kept in a shaker incubator for 24 hrs. Temperature was maintained at 37°C and the incubator was set to 150 rpm. The extract was filtered using a muslin cloth followed by Whatman no. 1 filter paper. The filtrate was evaporated in hot air oven at 50°C till dryness and residue was scrapped and stored at 4°C until further use.

Phytochemical screening

The aqueous extracts of *H. ferrugenia*, *H. grahamii*, and *H. nigra* were subjected to preliminary phytochemical screening following standard methods in order to find out the presence of following constituents (20-23).

Total phenolic content assay

The total phenolic content (TPC) of each extract was determined by Folin-Ciocalteu's colorimetric method as described earlier (24). Total phenolic contents were

expressed as mg of gallic acid equivalents per mg of the extract.

Total flavonoids content assay

The total flavonoid contents of the extracts were determined using the aluminium chloride assay (25). Total flavonoid contents were expressed as mg of quercetin equivalents per mg of the extract (QuE)/mg extract.

Total alkaloids content assay

The total alkaloid content of the extracts was determined using Harborne (1973) method (20). The alkaloid content was calculated in percentage.

Total saponin content assay

The total saponin content of the extracts were determined according to Nahapetian and Bassiri protocol (26). The saponin content was calculated in percentage.

Assessment of *in vitro* antioxidant activity

• Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was conducted according to Benzie and Strain method with slight modifications (27). Plant extract of test material in different concentrations ranging from 100µg to 500µg /ml were mixed with 2.5 mL of 0.2 mM phosphate buffer (pH 7.4) and 2.5 mL of potassium ferricyanide [1% weight/volume (W/V)]. The resulting mixture is incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10% W/V) and centrifuged at 3000 rpm for 10 minutes. Then, 2.5 mL of distilled water was added and later 0.5 mL of ferrous chloride (0.1% W/V). Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard.

• Phosphomolybdenum (PM) assay

The total antioxidant activity was estimated by phosphomolybdenum (PM) assay using the standard procedure of (28). Plant extract of test material in different concentration ranging from 100µg to 500µg/ml were added to each test tube individually containing 3 mL of distilled water and 1 mL of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 minutes. After incubation, they were kept at room temperature for 20-30 minutes and the absorbance were measured at 695 nm. Ascorbic acid was used as the reference standard.

• 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) assay

Free radical scavenging effect of plant extract was determined using the 2-diphenyl- 1picrylhydrazyl (DPPH) with slight modifications of the method described by (29). Briefly, the concentrations (100-500ug/ml) of extracts were prepared. DPPH solution

(0.004%) was prepared in ethanol and 1 ml of this solution was mixed with the same volume of aqueous leaf extracts and standard ascorbic acid solution separately. The mixture was kept for incubation for 30 minutes in the dark under room temperature and the optical density was measured at 517 nm. The level of DPPH purple decolorization to DPPH yellow confirmed the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

DPPH scavenging activity (%) = $\frac{AC-AT}{AC} \times 100$

Where AC- is the absorbance of the control reaction (1 ml of ethanol with 1 ml of DPPH solution), AT - is the absorbance of the test sample. The results were analysed in triplicate. The IC₅₀ value is the concentration of sample required to inhibit 50% of the DPPH free radical.

Statistical analysis

All experiments were performed in triplicate and the results were shown as mean \pm SD. The statistical significance for the biological activity assays was

evaluated using an ANOVA test with Tukey's Multiple Comparison by GraphPad Prism 5 program (ANOVA, p-values less than 0.05).

RESULTS

Qualitative and quantitative phytochemical analysis

The results of qualitative and quantitative phytochemical screening tests obtained from aqueous extracts of *H. ferrugenia*, *H. grahamii*, and *H. nigra* showed the presence of flavonoids, alkaloids, saponins and phenolic compounds (Table 1 and 2). The highest phenolic contents appeared in the aqueous crude extract of *H. nigra* followed by *H. ferrugenia* and *H. grahamii* extract. It was found that *H. ferrugenia* and *H. nigra* species contain higher amounts of flavonoids than *H. grahamii* species. All 3 species of Holigarna exhibited saponin content. It was also determined that *H. ferrugenia* species contained highest saponin compounds followed by *H. nigra* and *H. grahamii* whereas highest alkaloid content was seen in *H. nigra* when compared to *H. ferrugenia* and *H. grahamii*.

Table 1: Preliminary phytochemical analysis of aqueous extracts of *H. ferrugenia*, *H. grahamii*, and *H. nigra*

Phytochemical tests	<i>H. ferrugenia</i>	<i>H. grahamii</i>	<i>H. nigra</i>
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Glycosides	-	+	+
Cardiac glycosides	+	+	+
Terpinoids	-	+	+
Steroids	+	+	+
Phenols	+	+	+
Resin	-	-	-

+ = positive test; - = negative tests.

Table 2: Quantitative analysis of aqueous extracts of *H. ferrugenia*, *H. grahamii*, and *H. nigra*

	HN	HF	HG
Total alkaloid (mg/g)	15.033 \pm 0.208	5.306 \pm 0.136	5.8 \pm 1.2
Total saponin (mg/g)	10 \pm 2	25.466 \pm 0.702	3.8 \pm 0.6
Total Phenol (mg GAE/g extract)	0.526 \pm 0.011	0.410 \pm 0.014	0.233 \pm 0.009
Total Flavonoid (mg QE/g extract)	0.109 \pm 0.038	0.125 \pm 0.025	0.105 \pm 0.027

Values are mean of triplicate determination (n = 3) \pm standard deviation; GAE–Gallic acid equivalents; QE- Quercetin equivalents.

Physicochemical investigation revealed that moisture and total ash content was highest in *H. grahamii* whereas pH was found to be abundant in *H. nigra* while *H. ferrugenia* showed better acid insoluble ash property when compared with other species of same genus Holigarna. Similarly, elemental studies displayed the presence of various micro and macro

elements present in *H. ferrugenia*, *H. nigra* and *H. grahamii*. It was commonly seen in all plants that potassium was found in huge concentration compared to other elements while chromium, nickel and lead were below detectable level in all plants (Table 3 and 4).

Table 3: Physicochemical properties of *H. ferrugenia*, *H. grahamii*, and *H. nigra*

	HN	HF	HG
Moisture (%)	6.73 \pm 0.489	5.566 \pm 0.126	6.79 \pm 0.290
pH	4.70 \pm 0.210	4.476 \pm 0.251	4.56 \pm 0.383
Total Ash	6.09 \pm 0.290	6.433 \pm 0.351	6.75 \pm 0.317
Acid insoluble ash	0.466 \pm 0.061	0.513 \pm 0.015	1.47 \pm 0.226

Each value is presented as Mean \pm SEM (n=3)

Table 4: Estimation of micro and macro elements of *H. ferrugenia*, *H. grahamii*, and *H. nigra*

	HN	HF	HG
Sodium (mg/L)	6.826±0.338	6.650±0.595	3.45±0.0462
Potassium (mg/L)	74.67±3.486	62.706±1.311	59.24±0.2.35
Zinc (mg/L)	0.074±0.008	0.117±0.006	0.085±0.005
Iron (mg/L)	0.356±0.015	0.480±0.013	0.52±0.046
Chromium (mg/L)	BDL	BDL	BDL
Lead (mg/L)	BDL	BDL	BDL
Nickel (mg/L)	BDL	BDL	BDL

Each value is presented as mean±SEM (n=3); BDL – Below detectable level.

Antioxidant activity of plants

In this study, DPPH, FRAP and PM methods were used to analyse the antioxidant activity of *H. ferrugenia*, *H. grahamii*, and *H. nigra*. It was found that the aqueous crude extracts of *Holigarna grahamii* showed stronger FRAP activity compared to *H. nigra* and *H. ferrugenia* (Fig. 1). When the radical scavenging activities of the three plants were

compared, *H. nigra* species was found to have higher DPPH (IC₅₀ value: 128.8µg/mL) followed by *H. ferrugenia* (IC₅₀ value: 213.1 µg/mL) and *H. grahamii* (IC₅₀ value: 422.7 µg/mL) in this study (Fig. 2). PM activity showed good result for *H. nigra* as well followed by *H. ferrugenia* and *H. grahamii* (Fig. 3). In addition, ascorbic acid was used as standard in this study.

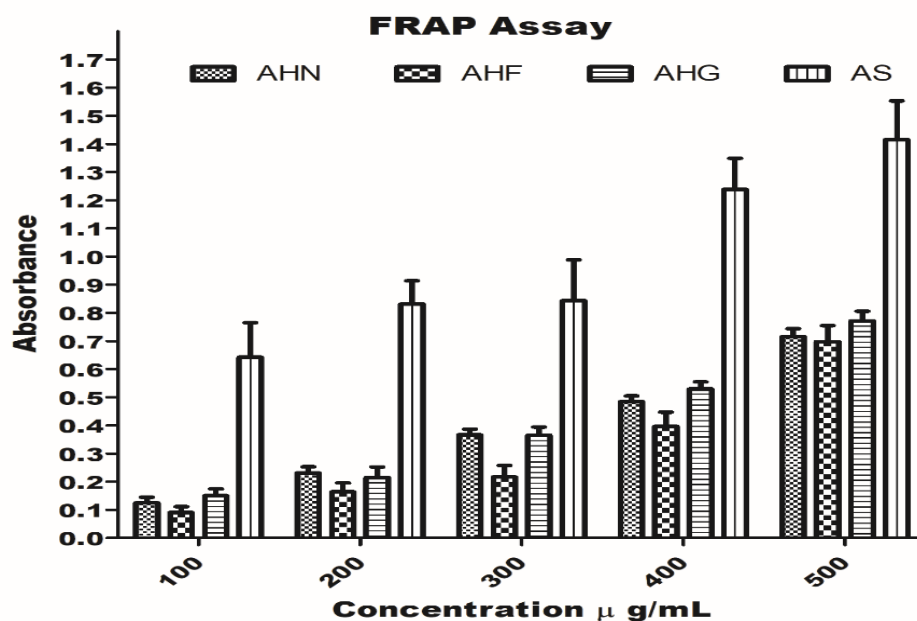


Fig. 1: FRAP activity of aqueous extract of root *H. ferrugenia*, *H. nigra* and *H. grahamii*. Each value is expressed as means ± standard deviation.

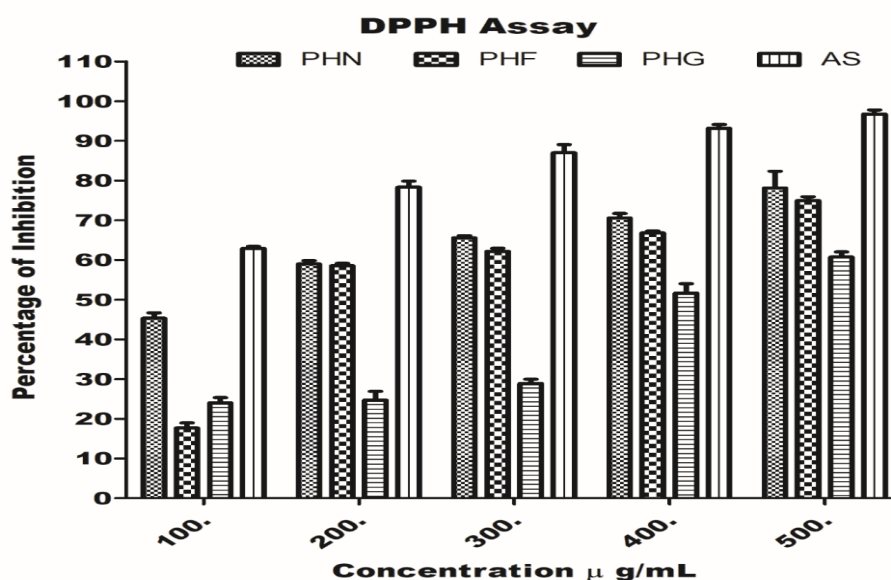


Fig. 2: DPPH scavenging activity of aqueous extract of root *H. ferrugenia*, *H. nigra* and *H. grahamii*. Each value is expressed as means ± standard deviation. Concentration (µg/ml) taken on x-axis and % inhibition taken on y-axis.

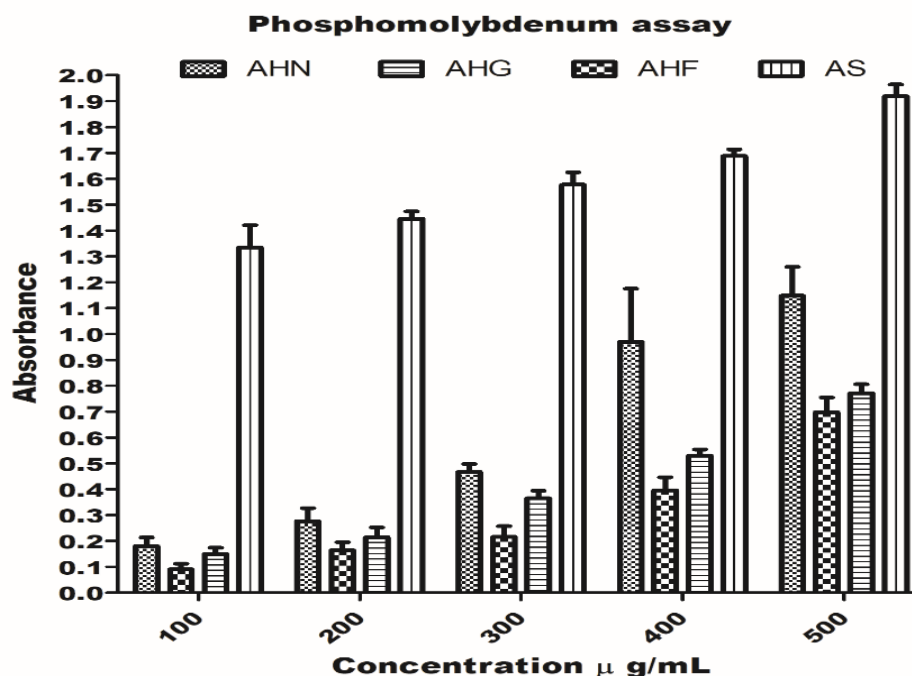


Fig. 3: PM activity of aqueous extract of root *H. ferruginea*, *H. nigra* and *H. grahamii*. Each value is expressed as Mean \pm standard deviation.

DISCUSSION

In recent years, herbal medicines have received large attention due to the presence of various phytoconstituents with multiple pharmacological properties. In the present study, Holigarna species showed the physicochemical and elemental properties. The secondary metabolites such as phenolic compounds, flavonoids, alkaloids act as antioxidants and scavenge free radicals as well as protect against oxidative degenerative diseases.

Oxidative stress is caused by an imbalance between free radical production and antioxidant defence and also plays a role in a variety of chronic diseases. Studies have shown that bioactive compounds provide protective effects against oxidative stress (30-32). The antioxidant activity of aqueous extract was evaluated by DPPH radical scavenging assay. The experimental data showed that all 3 species of Holigarna have good radical scavenging property and higher antioxidant capacity was found in *H. nigra*. The potential DPPH radical scavenging activity observed in this study may be due to the presence of these secondary metabolites in the extracts. These findings may mediate through any of the above signalling pathways (33). Our observation is in agreement with the studies of Izuegbuna *et al.*, (34). In a study by Johora *et al.*, the antioxidant activity of methanolic extract of *F. racemosa* was studied. They reported that the scavenging activity of *F. racemosa* from leaves and bark extracts was 48.54701% and 73.46154%, respectively (35). Antioxidant compounds originating from plants can reduce the formation of free radicals and can lessen the diseases initiated by oxidative stress (36). From the above results, it is observed that aqueous extracts of all 3 Holigarna species have good antioxidant properties. The flavonoids, alkaloids and

phenolics compounds obtained from remedial herbs might be responsible for antioxidative property of plants.

CONCLUSION

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Despite being tagged as toxic plant species, Holigarna species possesses a good quality of phytochemicals which directly or indirectly help in the health maintenance of living beings. In this study, the assessment of antioxidant activity indicates that wild leafy plants with higher phenolic and flavonoid contents could be a significant source of natural antioxidants. Although the parameters used in this study were not disease-specific, the quantification of antioxidant properties can serve as a guide for the use of these plants for ROS-related diseases. The selected plants with high antioxidant activity might be proposed for impeding toxic oxidation in nutraceuticals or drugs for the treatment of coronary diseases. Their anti-oxidant activity may be due to the phytochemicals present in the plants. Further work is required to fully elucidate the phytochemicals responsible for this action so that isolation and identification of responsible bioactive components will help in understanding their mechanisms of action, for future herbal drug formulations.

ACKNOWLEDGEMENT

Authors would like to thank Prof. B. K. Sarojini, Coordinator of Biochemistry Course, and Prof. Raju Krishna Chalannavar, Chairman of Applied Botany Department, Mangalagangothri, Mangalore

University for providing the laboratory facility to carry out the research.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

- Ambu, G., Chaudhary, R. P., Mariotti, M., Cornara, L. Traditional uses of medicinal plants by ethnic people in the Kavrepalanchok district, Central Nepal. *Plants*. 2020 Jun; 9(6): 759.
- Fang, Z. Research and Application of Medicinal Plants in Garden Landscapes. *Journal of Architectural Research and Development*. 2020 Oct 27; 4(5).
- Berdy, J. Bioactive microbial metabolites. *The Journal of antibiotics*. 2005 Jan;58(1): 1-26.
- Harsha, V. H., Hebbar, S. S., Hegde, G. R., Shripathi, V. Ethnomedical knowledge of plants used by Kunabi Tribe of Karnataka in India. *Fitoterapia*. 2002 Jul 1; 73(4): 281-287.
- Kubmarawa, D., Wase, G. A., Ayinla, O. G. Preliminary studies on phytochemical analysis and antimicrobial evaluation of extracts of *commiphora kerstingii*, *J. Chem. Soc. Nigeria*. 2007; 32(1): 38-40.
- Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., Nyirenda, C. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*. 2020 Sep 1; 9: e00481.
- Ravi, A., Saj, O. P. Antioxidant potential of bark extract of *Holigarna arnottiana* Hook. F. *Indian J Drugs Dis*. 2012; 1(2): 32-34.
- Škrovnáková, S., Mišurcová, L., Machů, L. Antioxidant activity and protecting health effects of common medicinal plants. In *Advances in Food and Nutrition Research*. 2012; 67: 75-139. Academic Press.
- Chopra, R. N., Chopra, I. C., Handa, K. L., Kapur, L. B. "Indigenous Drugs of India" U. N. Dhar and Sons Private Limited, Calcutta; 1958.
- Iyengar, M. A., Bhat, K. G., Nayak, S. G. K., Rajagopal, P. K. and Rajendra Singh. Survey of Medicinal Flora of South Kanara, Series III, *Indian Drugs*. 1987; 25 (2): 45 -51.
- Ravi, A., Saj, O. P. Antioxidant and cytotoxic potential of the plant *Holigarna arnottiana* hook. F. Bark ethanolic extract. *World Journal of Pharmaceutical Research*. 2015; 4(6): 1234-1244.
- Nayak, S. G., Kulkarni, S. B., Iyengar, M. A. Histology of the bark and leaf of a toxic plant *Holigarna ferruginea* march. *Ancient Science of Life*. 1993 Jan; 12(3-4): 327.
- Manilal, A., Idhayadhulla, A. Potential *in vitro* antimicrobial efficacy of *Holigarna arnottiana* (Hook F). *Asian Pacific Journal of Tropical Biomedicine*. 2014 Jan 1; 4(1): 25-29.
- Arambewela, L. S., Arawawala, L. D. Standardization of *Alpinia calcarata* Roscoe rhizomes. *Pharmacognosy Res*. 2010; 2(5): 285-288.
- Adeyeye, E. I., Agesin, O. O. Dehulling the African yam bean (*Sphenostylis stenocarpa* Hochst. ex A. Rich) seeds: Any nutritional importance? Note I. *Bangladesh J Sci Ind Res*. 2007; 42(2): 163-174.
- AOAC. Official Method of Analysis of Association of Analytical Chemists. 17th Edn., Association of Official Analytical Chemists (AOAC) International, USA. 2000.
- Odebiyi, O. O., Sofowora, E. A. Phytochemical screening of Nigerian medicinal plants II. *Lloydia*. 1978; 41(3): 234.
- Sunderman, F. W. Atomic absorption spectrometry of trace metal in clinical pathology. *Human Pathol* 1973; 4(4): 549-582.
- Kolthoff, I. M., Elving, P. J. Treatise on analytical chemistry. Vol. 15. New York, USA: John Wiley & Sons 1976; 15-100.
- Harborne, J. B. *Phytochemical Methods*. Chapman and Hall Ltd, London. 1973; 25-27p.
- Evans, W. Trease and Evans' Pharmacognosy. Elsevier Health Sciences, New York. 2002; 21-24.
- Debiyi, O. O., Sofowora, F. A. Pytochemical screening of medical plants. *Iloyidia*. 1978; 3: 234-246.
- Roopashree, T. S., Dang, R., Rani, R. H. S., Narendra, C. Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *Int. J. Appl. Res. Nat. Prod*. 2008; 1 (3): 20-28.
- Noreen, H., Semmar, N., Farman, M., McCullagh, J. S. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. *Asian Pacific Journal of Tropical Medicine*. 2017 Aug 1; 10(8): 792-801.
- Iqbal, E., Salim, K. A., Lim, L. B. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science*. 2015 Jul 1; 27(3): 224-232.
- Nahapetian, A., Bassiri, A. Changes in concentration and interrelationship of phytate, P, Mg, Cu, Zn in wheat during maturation. *Journal of Agricultural and Food Chemistry*. 1974; 32: 1179-1182.
- Benzie, I. F., Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*. 1996 Jul 15; 239(1): 70-76.
- Prieto, P., Pineda, M., Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*. 1999 May 1; 269(2): 337-341.
- Brand-Williams, W., Cuvelier, M. E., Berset, C. L. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*. 1995 Jan 1; 28(1): 25-30.
- Jeon, J., Sung, J., Lee, H., Kim, Y., Jeong, H. S., Lee, J. Protective activity of caffeic acid and sinapic acid against UVB-induced photoaging in human fibroblasts. *J. Food Biochem*. 2019; 43 (2): e12701.
- Sim, U., Jeehye, S., Hana, L., Huijin, H., Heon, S. J., Junsoo, L. Effect of calcium chloride and sucrose on the composition of bioactive compounds and antioxidant activities in buckwheat sprouts. *Food Chem*. 2020; 312: 126-127.
- Taskin, T., Dogan, M., Yilmaz, B. N., Senkardes, I. Phytochemical screening and evaluation of antioxidant, enzyme inhibition, anti-proliferative and calcium oxalate anti-crystallization activities of *Micromeria fruticosa* spp. brachycalyx and *Rhus coriaria*. *Biocatalysis and Agricultural Biotechnology*. 2020 Jun 2: 101670.
- Usha, B., Karanth, J., Joshi, C. G. Antioxidant, anti-inflammatory, analgesic and anti-proliferating activities of *Grewia heterotricha* Mast. *Biomedicine*. 2020 Nov 9; 40(3): 272-277.
- Izuegbuna, O., Otunola, G., Bradley, G. Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of *Opuntia stricta* cladodes. *PLoS One*. 2019; 14(1): e0209682.
- Johora, S., Kabir, A. S., Abdul, H., Abdullah, M., Islam, N., Abu, R. Evaluation of the antioxidant activity of *Ficus racemosa* plant extracts from northwestern district of Bangladesh. *J Life Earth Sci*. 2013; 8: 93-99.
- Rastogi, S., Iqbal, M. S., Ohri, D. *In vitro* study of anti-inflammatory and antioxidant activity of some medicinal plants and their interrelationship. *In vitro*. 2018; 11(4).