JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANTS PROPERTIES OF *MYRICA NAGI*-A HIMALAYAN JEWEL

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Received: April 2020 Revised and Accepted: June 2020

ABSTRACT: *Myrica nagi*, popularly known as Kafal in Ayurveda is a crown of wild Himalayan region and used traditionally to cure several ailments. This study attains to understand the preliminary phytochemical analysis and antioxidant activity of different solvent (water and methanol) extracts of *Myrica nagi*. The preliminary phytochemical screening revealed the existence of alkaloids, flavonoids, glycosides, saponins, sterols, and tannins in both of extracts. Antioxidant activity was done using standard scavenging assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH), FRP and H_2O_2. The result indicated that methanol as the most potent solvent for polyphenols extraction. The methanol extract found to have higher phenolic content and flavonoid content (95 ± 8.14 mg of equivalent gallic acid (GAE)/g; 118.74 ± 6.41 mg quercetin equivalent (QE)/g)) than the aqueous extract (89.01 ± 8.26 GAE/g; 35.77 ± 0.14 QE/g), respectively. The methanol extract of *M. nagi* demonstrated the highest DPPH scavenging activity (IC_{50} 29.62 µg/mL), at the same time the aqueous extract (IC_{50} 22.54 µg/mL) showed minimum antioxidant impending. To conclude, these results suggested that the methanolic extract from *M. nagi* leaves found to have more antioxidant prospective and serving a considerable basis of natural antioxidants for the traditional formulation.

KEYWORDS: *Myrica nagi*, antioxidant, extracts, methanol

I. INTRODUCTION

Without oxygen, survival of human life is impossible. But, it can affect human beings by its different forms like superoxide anion, hydroxyl radical, hydrogen peroxide etc. known as ROS (Reactive oxygen species). It causes diseases such as asthma, dementia, carcinoma, arthritis and Parkinson's disease [1]. Normally, at small quantity these ROS and Reactive Nitrogen species (RNS) shows necessary physiological activities namely metabolism, programmed cell death, transportation of solutes etc. But, when the concentration exceeds the optimum, the risk factors also increases and it can cause various incurable diseases in humans like chronic inflammatory reaction, auto immune and neurodegenerative diseases, cardiovascular disease, diabetes, cancer [2,3].

Antioxidant is the best precaution taken for the above mentioned pathological conditions. It is the direct scavenger of ROS and RNS [2, 4]. Besides that, isolation of secondary metabolite having antioxidant property is one of the hot topics of the current research era. So, screening of medicinal plants is valuable for their antioxidant properties. The medicinal plants have been formulated to treat different diseases all over the world since time immemorial [5,6,7]. Now-a-days scientists are more attentive towards the natural products to synchronize their potentiality with traditional system of medicine and their scientific validation [7,8,9].

Most of antioxidant activity of different phytochemicals in nature is found in polyphenols [10]. However, polyphenols can change their properties while, processing of food and storage, where it can directly affect the stability of food. But in this era, people are facing a huge problem with synthetic antioxidants and that gave a chance to think about natural antioxidants [11]. So, plant derived phytochemical is the only hope for the adequate antioxidant in the body. Connecting to the present perspective, the aim of the study is to understand naturally occurring antioxidants and their scavenging activity of *Myrica nagi* which can further show a huge impact on the modern system of medicine by its medicinal properties.

Myrica nagi also called *'Box myrtle',* a sub temperate evergreen tree. It is widely distributed all over the mid Himalayas, Khasia hills, Sylhet and southwards up to Singapore, Malay island and in China and Japan [12]. This evergreen and dioecious plant is a medium to large woody, 12-15 meters in height. Leaves are crowded

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towards the end of branches, having 9.2cm long lanceolate. *Myrica* is most popular for its juicy fruit products [13].

Myrica has a good history for combating various diseases represented in classical system of medicine [2,12,14]. The bark, flowers, fruits and roots of *Myrica* is used as medicine in Ayurveda and Unani system. According to Patel *et al, Myrica* bark extract shows potent anti-allergic activity on mice [15]. Impressive anti-inflammatory activity was observed in ethyl acetate and aqueous extracts of *Myrica* bark [16]. Middha et al., 2016 also indicated the anti-inflammatory activity of the *Myrica nagi* in methanolic extract [12]. Jain *et al.,* in their study proved the higher anti-helminthic activity with *Myrica's* ethanolic extract (50%) on *P. posthuma* species [17]. *Myrica* acts as a persuasive chemo preventive agent on skin by reducing the induced toxicity caused by the cutaneous oxidative stress (COS) [18]. *Myrica* bark oil has a potent anti-microbial activity [19]. Our present investigation is to evaluate the antioxidant activity by employing different *in-vitro* antioxidant models.

II. MATERIALS

Plant material and extraction

The collection source of leaves for *Myrica nagi* Thunb. were NEHU, Shillong, Meghalaya (25.6121° N, 91.8977° E) in 2016 and were authenticated, certified by Dr. AK Misra Kumar, Taxonomist, NEHU. The specimen was submitted (MLAC/MN05) to Botany department, MLACW under Bangalore University, Bengaluru, Karnataka.

Preparation of Extracts:

Dried, washed and ground leaves of *M. nagi* were extracted by Soxhlation using 1:15 *w/v* the ratio of plant material to two different solvent methanol (ME) and water [20]. The aqueous (AE) and methanolic extractions were done for 6 hours for three times at their boiling temperatures. The extracts obtained were filtered, evaporated while hot using rotary evaporator. The extract was stored at low temperature at deep freezer for further use. The residues or extract was dissolved in sterile double distilled water in appropriate concentration just prior to use. The yields of final evaporated extracts were calculated using standard equation as previously provided by Middha et al., 2015 [20].

$$\text{Yield (gm /100 gm of dry plant material)} = (W_1 / W_2) \times 100$$

Preliminary Pl

The phytochemical screening was carried out for the *M.nagi* leaf extract to find the presence or absence of different constituents with medicinal importance. Different phytoconstituents namely Carbohydrates, Reducing sugars, Tannins, Saponnins, Flavonoids, Steroids, Alkaloids, Anthraquinones and Glycosides were analyzed using standard method as mentioned in Goyal et al., 2010 [21].

Determination of Total Flavonoid content (TFC)

The TFC content of both extracts (AE and ME) were determined with standard Aluminium-chloride colorimetric method ($AlCl_3$) using a standard quercetin [22]. To 1 mg/ml of extract (0.1ml), add double distil water and 5% $NaNO_2$ solution. Incubate for 5mins at room temperature(RT). Add 10% AlCl3 solution and incubate at RT for 6mins. Then add,1M NaOH solution and dilute with distilled water. The final absorbance (OD) was recorded at 510 nm.

Determination of Total Phenolic Content (TPC)

The TPC content of both extract (AE and ME) were determined by Singleton and Rossi method using Folin-Ciocalteau(FC) reagent [23]. 1mg/ml of the extract (0.1ml) was thoroughly mixed with 0.5ml of FC reagent for 5mins at RT. Then add 20% (w/v) of sodium carbonate and incubate it at RT for 10mins. The blue color solution was read at 760nm to find the absorbance. In this assay standard used is gallic acid. The concentration of TPC in gallic acid was read as µg of gallic acid equivalent (GAE).

In-vitro Antioxidant activity of *Myrica* extract

Free Radical Scavenging Activity using DPPH method

The scavenging property of the *Myrica* extract and standard was estimated by the action of the stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in scavenging effect of the free radicals as reported by Goyal *et al.*2010

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[21]. ME and AE were The used concentrations of ME and AE were varying from 20 to 100 µg/mL. 50 µM $DPPH^{\cdot}$ solution was mixed to the various plant extracts concentrations. The standard ascorbic acid (AA) was also prepared in similar fashion. The mixtures were kept at a dark place for half an hour. The 2 ml of methanol and DPPH solution (equal volume) were used as a control solution. All the extract mixtures along with control solution were quantified at 517nm using a double beam spectrophotometer (Systronics 2021). The percentage inhibition or DPPH scavenging activity was calculated by the mentioned formula.

$$DPPH\ scavenging\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where, $\mathbf{Abs_{control}}$**:** Absorbance of the control and $\mathbf{Abs_{sample}}$**:** Absorbance in the presence of the sample.

IC_{50} value is also determined using the graph plotted, which is the concentration of the extract required to scavenge 50% of the free radical present.

Scavenging of Hydrogen Peroxide (H_2O_2):

The potential of the extracts (AE and ME) to scavenge H_2O_2 was evaluated as previously experimented by Goyal et al., 2013 [24]. The percentage scavenging of H_2O_2 by both of the extracts were calculated using the below mentioned formula,

$$H_2O_2\ scavenging\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where,

$\mathbf{Abs_{control}}$**:** Absorbance of the control and $\mathbf{Abs_{sample}}$**:** Absorbance of the extract

Ferrous Reducing Power Assay (FRP):

The FRP of the extracts (AE and ME) was found according to the Oyaizu method [25]. The activity was measured at 700nm and then compared with the standard. Increase in the absorbance (OD) of the reaction mixture indicated elevated reducing power of the extract.

Statistical Analysis

All the Results are shown as mean ± Standard error of mean (in triplets). Statistical analysis was carried out using Student's t-test analysis and correlation using Microsoft excel tool. The tests were considered significant at $p<0.05$.

III. RESULT AND DISCUSSION

Plant Extract Yield

14.72 gm of plant powder was obtained after Shade drying that was used for further extraction. 0.65 gram of extract was obtained by soxhlation,using methanol as solvent (ME), giving 4.41% of total powdered sample. The yield of aqueous extract (AE) of *Myrica* leaf residue was 2.19 %.

Preliminary Phytochemical Screening *Myrica* extract

Preliminary phytochemical screening of the ME and AE extract of *Myrica* leaf extract witnessed the presence of numerous bio-active compounds such as alkaloids, carbohydrates, glycosides, steroids, saponins, resins, tannins and phenols were explored using standard methods. The results indicated that AE and ME have alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, steroids, and tannins (Table I). Some of the reports in other plants have shown flavonoids and phenolic compounds are correlated with the anti-oxidative property in biological systems. [26, 27]

Table I: Preliminary phytochemical screening of *Myrica nagi* extracts

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Phytochemicals	ME	AE
Alkaloids	-	+
Carbohydrates	+	-
Glycosides	+	-
Flavonoids	+	+
Saponins	+	+
Steroids	+	-
Tannins	+	-
Phenols	+	+

*(+) Presence (-) Absence, whereas ME, Methanolic extract of *Myrica;* AE, Aqueous extract of *Myrica*

Total Flavonoid content (TFC)

Flavonoids are the molecules found as secondary metabolites in plants. Potency of a flavonoid depends on the position and number of free -OH groups [28]. Authors have used $AlCl_3$ method to detect flavonoids in the selected plant extract. The TFC of the ME and AE was found to be 118.74±6.41 mg quercetin/gm (QE)/g) and 35.77 ± 0.14 mg quercetin/gm (QE)/g). The results were calculated a graph plotted using QE as a standard ($y=0.004x + 0.351$, $R^2= 0.968$). The outcome indicated the ME displayed higher flavonoids content than AE. Methanol is used extensively to extract phytochemical [20, 21, 24] even in the case of *Myrica* as reported by several studies [12, 28]. Flavonoids are the major phenolic group and have a major role in color and flavor to the vegetables and fruits [29]. Richness in flavonoids also indicated the high free radical scavenging and seen below as DPPH activity.

Total Phenolic Content (TPC)

Folin–Ciocalteu reagent method is a standard procedure used to understand the total phenolic compounds in the extracts. These compounds act as a potential antioxidants, having redox activity [30]. The TPC of ME and AE was 95.10±8.24 mg equivalent gallic acid (GAE/gm) and 89.01±8.26 mg GAE/gm of dry extract. TPC was calculated based on the linear equation drawn on the gallic acid curve ($y=0.007x+0.495$, $R^2=0.969$).

The higher content of phenolic acid proportionates the high antioxidant properties and bioactivity of the extract [12, 20]. Methonolic and aqueous extract also reciprocates the same in our study. In their previous studies, several reports also advocated the methanolic extract being the better solvent to extract better phenolic compounds [12, 28]. Though, it depends on the interest of the study and compound of interest as well as their solubility. Many previous studies have shown that biological molecules are damaged by the generation of free radicals under oxidative stress conditions [31, 32]. Parallel research work also has reported very similar results elsewhere [7,11,12,26]. Misra et al also reported a relationship between TPC and antioxidant properties [27].

***In-vitro* Antioxidant activity of *Myrica* extract**

Free Radical Scavenging Activity

The DPPH scavenging activity is widely used to detect antiradical activity. In this assay, radical scavenging activity enhanced with increase in concentration of extracts. ME fraction exhibited significant scavenging activity when compared to AE fraction. Highest radical scavenging activity may be due to its high phenolic acids. At the highest concentration of 1000µg/ml ME fraction had the highest scavenging activity of 83.49% followed by AE extract (74.3%) whereas, standard AA showed the lowest scavenging activity (Figure 1). The scavenging activity of ME may be highest in ME, may be because of synergetic effects of high phenolic compounds present in the same. The methanol extract of *M. nagi* demonstrated the highest DPPH scavenging activity (IC_{50} 29.62 µg/mL), at the same time the aqueous extract (IC_{50} 22.54 µg/mL) showed minimum

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antioxidant properties (Table II). The basic principle of DPPH assay is absorbance of deep purple colour complex at 540nm. The decolorization of DPPH would have occurred with the help of odd electron present on the DPPH molecule in the existence of anti-oxidants. As and when DPPH accepts an electron from antioxidant molecule, it is decolorized and is measured based on the change in the absorbance [20]. Appreciable difference in DPPH scavenging activity of *Myrica* leaf extracts were observed in our study. Data from our research work, affirm that the ME has high effects on -OH scavenging matching with the earlier studies [12]. Flavonoids like myricetin may be accountable for the anti-oxidative effects [14]. The antioxidant property of flavonoids and phenolics are primarily due to their redox mechanism, that plays a vital role in quashing triplet and singlet oxygen, nullifying free radicals and disintegrating peroxides [29].The polyphenol compounds naturally found in plants have been recorded to have various pharmacological effects including antioxidant property [30,31]. Comparative analysis of the ME and AE with free radical scavenging activity and standard AA is shown in figure 1. Several reports, previously, have also indicated that antioxidant activities are directly connected with both TFC and TPC [26,27].

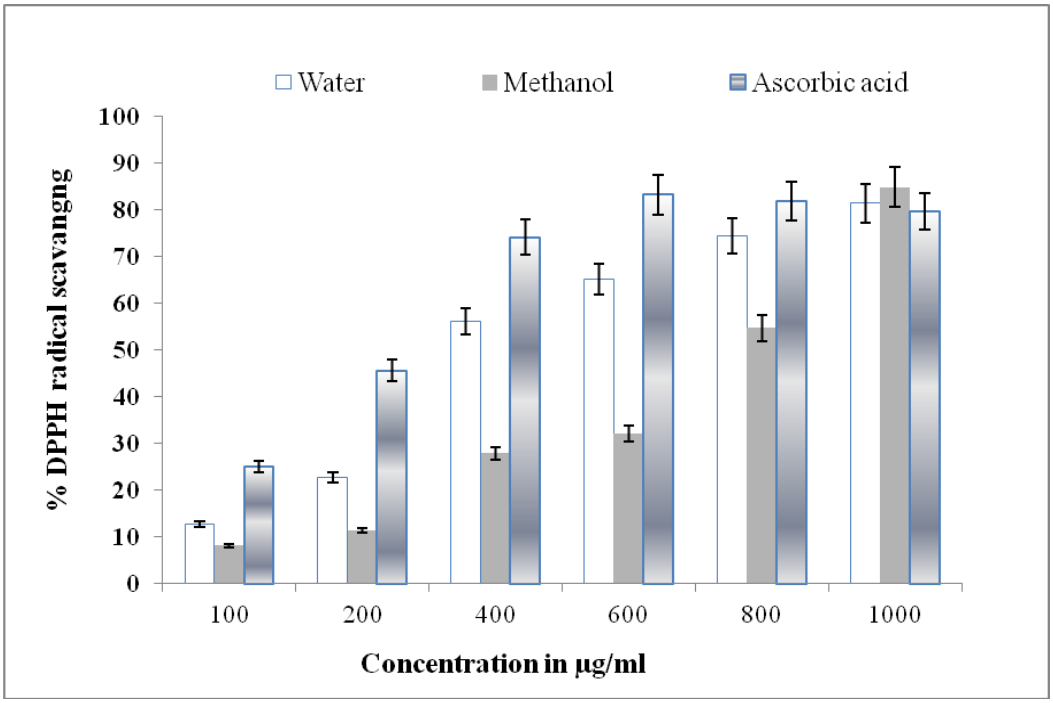

Figure 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of leaf extracts.

Table II. IC_{50} of *M. nagi* extracts in DPPH antioxidant assay.

Crude Extracts	DPPH Assay (µg/mL)	H_2O_2 (µg/0.1mL)
Aqueous	22.54[a]	13.78[a]
Methanol	29.62[b]	16.97[b]
Ascorbic acid	34.26[c]	23.23[c]

***Statistical significance was calculated at $p < 0.05$ and is specified with different letters**.

Scavenging of Hydrogen Peroxide

H_2O_2 scavenging activity of ME, AE and standard AA is depicted in figure 2 which shows a high amount of scavenging activity. H_2O_2 is known to be significant because of its capability to infiltrate into biological membranes. It is highly toxic to cells because of its ability to produce or rise to hydroxyl (-OH) radicals inside the cells [26,27]. The results showed the extracts had potent H_2O_2 scavenging activity which could be due to the phenolic antioxidant compounds. ME extract showed better scavenging ability as compared to AE extract. As the concentration rise the scavenging percentage of H_2O_2 were also seen increasing. The methanol extract of *M. nagi*

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demonstrated the highest H_2O_2 scavenging activity (IC_{50} 16.97 µg/0.1 mL), at the same time the aqueous extract (IC_{50} 13.78 µg/0.1mL) showed minimum antioxidant properties (Table II).

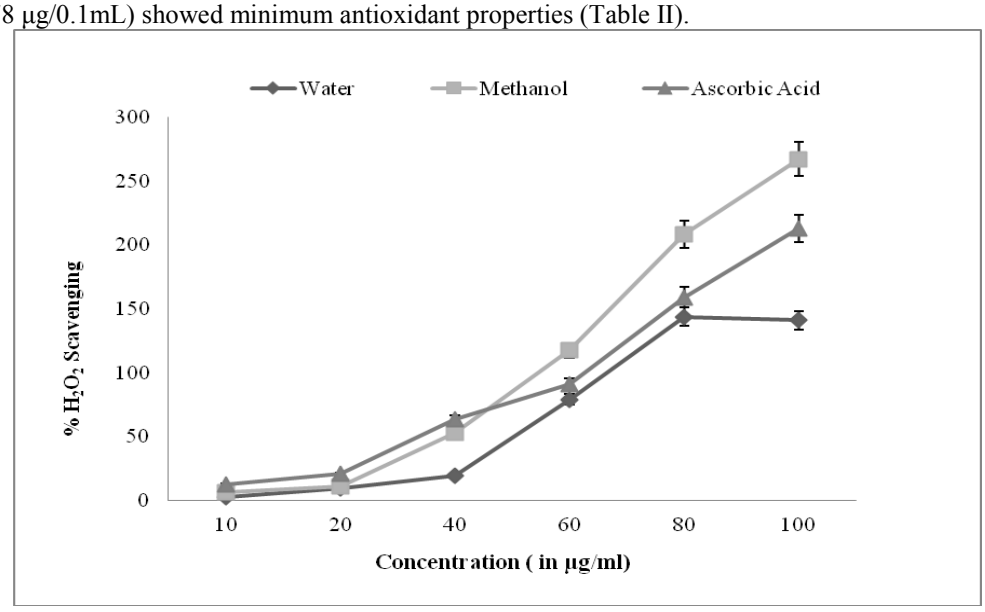

Figure 2. H_2O_2 scavenging activity activity of different leaf extracts of *Myrica*.

Several phenolic compounds, anthocyanins, and tannins in natural products have found a immense significance due to their affluent antioxidant potentials. [33,34,35]. Interestingly, several studies documented that due to their redox properties, theses complexes also provide as a potent reducing mediators, hydrogen givers, singlet oxygen inhibitors and effective metal chelators [33, 36]

Ferrous Reducing Assay (FRP)

Reducing capability (RC) of ME is compared with AA and been depicted in figure.3. FRP assay is used to quantify the reducing latent of an antioxidant. Figure 3 witnessed the measure of RC showing Fe^{3+} transforming to Fe^{2+} in the presence of ME, AE and AA. The RC of the extracts ME and AE were found to be increased gradually with an increase in concentration of ME and AE. It is evident from the figure 3 that ME extract showed the highest reducing capabilities when compared with the standard AA and AE extract. The maximum reducing power was found at 100µg/ml of ME, AE and AA. The reducing power of ME was found to be more appreciable that increased exponentially with the increase in concentration [26, 27]. From this, it's proved that a low dose of ME had maximum reducing power as compared with the standard AA. This might be ME extract found to have more phenolics than AE extract. It has been seen that the Fe^{3+} ions reduced to Fe^{2+} in the existence of the polyphenols in ME proving the potential of reducing power of the extract [20]. Previously, similar kind of results have been recorded with some plant product deploying ME extract [20,26,28].The absorbance of ME and standard AA were found similar at 20 µg/mL concentrations. A direct statistical correlation between reducing power and antioxidant property of plant extracts have been studied by many scientists in parallel [26, 27].

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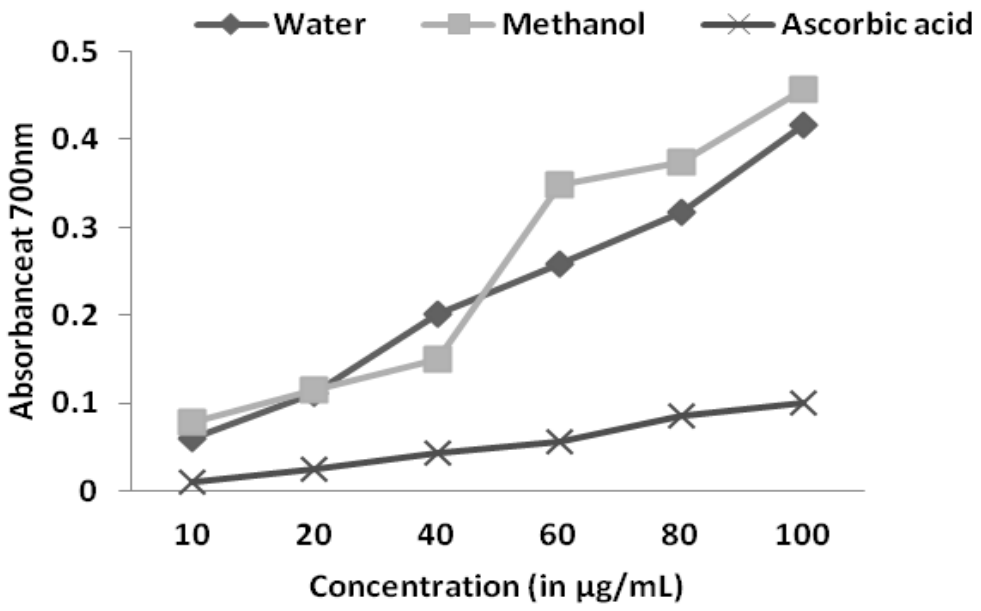

Figure 3. Ferrous reducing activity of different leaf extracts of *Myrica*.

IV. CONCLUSION:

This investigation indicated that *M. nagi* leaf extracts have significant antioxidants demonstrating to their richness in phenolics and flavonoids. The methanolic extract was the found to be rich in TPC and TFC as compared to aqueous extract. The free radical activity of the different *Myrica* extracts understood to be strongly co-related with TFC and TPC contents. To conclude, isolation, purification and characterization of individual bioactive compounds are further needed in search of new lead with regards to human ailments.

Author Contributions: Conceptualization, S.K.M.; methodology, H.P.P. and S.D.; software, R.N.; validation, H.P.P.; formal analysis, S.D.; investigation, H.P.P.; resources, S.K.M.; data collection, H.P.P; writing—original draft preparation, H.P.P and S.D..; writing—review and editing, S.K.M. and R.N.; supervision, S.K.M. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Acknowledgments: Authors are greatful to Management, Maharani Lakshmi Ammanni College for Women, Bengaluru for providing lab facilities and chemicals.

Conflicts of Interest: The authors declare no conflict of interest

V. REFERENCES:

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