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(RESEARCH ARTICLE)

In-vitro evaluation of antioxidant properties of aqueous extracts of *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis*

Nupur Sinha * and Rahul Ellur

Acharya Institute of Allied Health Sciences, Acharya Institutes, Bengaluru. 560107, India.

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Abstract

This study investigates the antioxidant properties of aqueous extracts of *Murraya koenigii* (curry leaf), *Aegle marmelos* (bael) and *Laurus nobilis* (bay leaf) using in vitro assays. The plants have been traditionally used in Indian medicinal systems for the treatment of various metabolic disorders. Aqueous extracts from the leaves of *Murraya koenigii,* fruit pulp of *Aegle marmelos* and leaves of *Laurus nobilis* were prepared. The total phenolic content and total flavanoid content was also determined in these aqueous extracts. They were tested for their ability to scavange free radicals by DPPH assay, ABTS assay and FRAP assay which are elevated during various metabolic disorders. The results suggest that all the three plants exhibited significant antioxidant activities. But strongest antioxidant properties were exhibited by *Murraya koenigii* leaf extract followed by the pulp of *Aegle marmelos* and lowest antioxidant activity was exhibited by *Laurus nobilis* extract, supporting their use in traditional medicine and potential development into therapeutic agents. So, this research suggested that these medicinal plants possess a significant antioxidant potential and are important source of natural antioxidants and can be effectively used in treating oxidative stress disorders. As a result, the study's significant findings demonstrate that it is a step towards evidence-based phytomedicine.

Keywords: *Murraya koenigii*; *Aegle marmelos*; *Laurus nobilis;* Antioxidant activity; DPPH assay; ABTS assay; FRAP assay

1. Introduction

The reactive oxygen species (ROS) are free radicals like hydroxyl radical, nitric oxide radical, hydrogen peroxide, and superoxide anion radical, hypochlorite radical, lipid peroxides, and various singlet oxygen molecules [1]. During the metabolic activities, our body produces certain compounds called free radicals by its own [2]. The normal physiological processes result in lesser production of free radicals in the body, but abnormal functioning or less availability of antioxidant level in the body results in oxidative stress resulting in the generation of these free radicals in high level [3]. These free radicals are the cause of many degenerative and chronic diseases like Parkinson's disease, arthritis, cancers, stroke, Alzheimer's disease, immune suppression, atherosclerosis, ageing, diabetes mellitus, chronic inflammatory diseases, ischemic heart disease, and neurodegenerative diseases [4]. On the other hand, some other factors i.e. alcohol, smoking, ionizing radiations, chronic diseases, and environmental pollution are also responsible for the increased level of these free radicals, in addition to natural causes [5].

These free radicals are bound by very vital chemical compounds, the antioxidants, which reduce and prevent us from the harmful effects on normal cells of the body. Some antioxidants produced artificially, such as butylated hydroxyl-toluene and butylated hydroxyl-anisole, which are available commercially are less stable and are very harmful, whereas natural antioxidants are safe with minimal side effects. Due to safety reasons, antioxidants have been preferably obtained from natural products [6]. Fruits, vegetables, seeds, herbs, sprouts, edible mushrooms, and cereals are the

* Corresponding author: Nupur Sinha

natural sources of food which can be used as an efficient source of these antioxidants to minimize the side effects and damage from free radicals [7]. The chemical substances present in plants, which have defensive effects, are called phytochemicals. The phytoconstituents found in the plants, which are responsible for antioxidant potential, are mainly flavonoids, phenols, anthocyanin, iso-flavones, flavones, lignins, catechins, iso-catechins, and coumarins. These phytochemical constituents are mainly determined by measuring total phenolic contents (TPC) and total flavonoid contents (TFC), and the antioxidant effects are determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), and 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS) assays [8].

Different medicinal plants having antioxidant potential have been efficiently applied to treat ROS and are of great importance because of their alimentary radical scavenging diet supplement. The antioxidant potentials of medicinal plants are mainly because of rich source of phyto-nutrients and ingredients like phenols, flavonoids, and terpenoids present in them. The antioxidant potentials of many medicinal plants have been studied for anti-cancer activity, immunomodulator activity, hepatoprotective effects and hypolipidemic activity [9]. In our study three plants were used namely, *Murraya koenigii* (curry leaf), *Aegle marmelos* (bael) and *Laurus nobilis* (bay leaf).

1.1. Murraya koenigii

Known by several Indian dialects as curry leaf or curry veppila, it is a member of the Rutaceae family. This little tree or shrub, which grows at a height of 4–6 meters, has a potent scent and lots of shade. *Murraya koenigii* is a native of tropical Asia, although it can be found in India and other countries. This plant's leaves are utilised in many curries throughout South India as a natural flavour.

Furthermore, the Indian ayurvedic system has long employed these leaves to treat diabetes. The plant is used to treat body heat, blood disorders, diarrhoea, dysentery, eruptions, inflammation, itching, kidney pain, leukoderma, piles, snakebite, thirst and vomiting. It also has acrid, analgesic, bitter, cooling, alexiteric, anthelmintic, carminative, purgative, and stimulant qualities. It is used as a blood cleanser, tonic, stomachic, antiemetic, antidiarrheal, dysentery, febrifuge, and flavouring agent in curries and chutneys in traditional medicine [10, 11]. Because plants contain physiologically active substances called phytoconstituents, the use of plant-based medicine has long been a pillar of traditional societies' approaches to treating health conditions [12, 13].

1.2. Aegle marmelos

A member of the Rutaceae family, commonly called Bael in Bangla, is a tree that grows in Bangladesh, India, and Southeast Asia. Many bioactive substances have been extracted from the plant, including tannin, aegeline, lupeol, cineole, citral, citronella, cuminaldehyde, eugenol, marmesinin, marmelosin, luvangetin, aurapten, psoralen, marmelide, fagarine, and tannin. Various parts of A. marmelos have been found to have therapeutic uses in the past. These include the treatment of intestinal ailments, asthma, anaemia, fractures, wound healing, swollen joints, high blood pressure, jaundice, diarrhoea, intermittent fever, and fish poisoning [14, 15]. *A. marmelos* is also used in Indian, Bangladeshi, and Sri Lankan traditional medical systems as a traditional herbal remedy for diabetes mellitus. The unripe dried fruit has stomachic, digestive and astringent qualities that make it useful in treating diarrhoea and dysentery. After recovering from bacillary dysentery, patients can benefit from a sweet drink made from fruit pulp [16-18].

1.3. Laurus nobilis

Bay leaf, also known as laurel leaf, is produced by the sweet bay tree (*Laurus nobilis*), an evergreen member of the Lauraceae family that is indigenous to areas bordering the Mediterranean. Apart from their significant health benefits, Indian bay leaves also serve as a rich source of vitamins, minerals, and other essential nutrients. These leaves contain a variety of essential nutrients, including ferrous, manganous, calciferous, cellulose, saccharides, ascorbic acid, cupric, pyridoxine, folic acid, and zinc [19]. Bay leaves have numerous biological properties that make them useful for various purposes. They have wound-healing abilities [20], act as antioxidants, and have antibacterial [21, 22], antiviral, and immunostimulant properties [23]. Additionally, they possess anticholinergic, antifungal [24] and insect-repellent properties, making them versatile for different applications. They also have anticonvulsant and antimutagenic effects and can act as analgesics and anti-inflammatory agents [25]. It can be applied to alleviate paralysis, convulsions, neuropathic pain, nerve entrapment, swelling and bruises, migraines, ear aches, and joint inflammation [26].

Our research aims to systematically validate the antioxidant properties of *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis* by DPPH, FRAP and ABTS Assay. Total phenolic content (TPC) and total flavonoid content (TFC) were also quantitated from these plant extracts to ensure their efficacy and safety in traditional healthcare applications.

2. Materials and Methods

2.1. Collection of Plant Material

The fresh leaves of *Murraya koenigii,* pulp of *Aegle marmelos* and dried leaves of *Laurus nobilis* were collected from the herbal garden.

2.2. Preparation of aqueous plant extracts

The preparation of plant extracts for each assay was carried out in compliance with the published protocol [27] with some modification. The collected plants were washed thoroughly to remove debris and were shade dried. These samples (parts used from each plant) were then grinded into fine powder after drying in an oven for 7 days. For preparation of aqueous extracts, the dried and powdered plant samples of about 100 g each were taken in conical flasks and was extracted with 100 mL of dH_2O using a stirrer. The filtration was achieved using Muslin cloth, the solution was then centrifuged, and rotary evaporator was used for sample drying through evaporation. Air-tight plastic vials were used to store the collected dried aqueous plant extract samples for further studies.

The powdered samples were subjected to quantitative phytochemical estimation of TPC and TFC, and antioxidant evaluation of these samples was performed by various antioxidant assays using spectrophotometer. Three antioxidant assays (DPPH, FRAP and ABTS assays) were used to determine the free radical scavenging potentials of these medicinal plants under study.

2.3. Determination of TPC

For estimation of TPC, Folin–Ciocalteu reagent was used to measure the TPC of these samples at 765 nm using UV spectrophotometer [28]. Stock solutions of each extract were prepared. The dilutions of aqueous extract of all three plant samples (0.5 mL of 1 mg/mL) and standard phenolic compound (gallic acid) using dH_2O (5 mL, 1:10 dilution) were made and then mixed with Folin–Ciocalteu reagent solution, adding 4 mL of 1 M aqueous sodium carbonate. The absorbance was measured at 765 nm after the mixture was kept for 30 min for total phenol estimation using spectrophotometer. A common reference compound, gallic acid equivalent (GAE; mg/g of dry mass) curve was used to express the TPCs. All experiments were conducted in triplicates and the data were analysed as average of three values.

2.4. Determination of TFC

Shinoda test: 4 mL of extract solution, 1.5 mL of 50% methanol solution, and a small magnesium chunk were warmed. On adding 5–6 drops of concentrated HCl, red color was observed for flavonoids [29]. To 10 mL volumetric flasks containing 4 mL of water, different plant extracts (1.0 mg/mL) and various dilutions (10–1,000 µg/mL) from Rutin (Standard) were added. Then, 0.3 mL of 5% $NaNO_2$ was added to the above mixture. 1 M NaOH (2 mL) was added after 6 min and 10 mL total volume was made by using dH_2O. The solution was mixed well, and absorbance was taken at 510 nm. The Rutin equivalent (mg/g of dry mass) a common reference compound was used to express the TFCs. All experiments were performed in triplicates and the data were analysed as average of three values.

2.5. *In-vitro* Antioxidant assays

To determine the antioxidant effects of plant extracts, different antioxidant assays for the selected plant extracts were performed according to standard procedures with minor modifications.

2.5.1. DPPH antioxidant assay

DPPH is a stable free radical chemical with purple color that absorbs at 517 nm. The DPPH purple color is converted to yellow color or even colorless if plant sample possess any potential free radical scavenging property. The antioxidant or radical scavenging properties of various plant samples were investigated by applying DPPH antioxidant assay with some modifications in the procedure adopted by Roberta et al. and Blois protocols [30, 31].

About 1 mL of test solution of aqueous plant extracts were dissolved in equivalent amount of DPPH solution (0.1 mM). The increase in DPPH absorbance of tested samples was measured after 20 min incubation at room temperature, by taking the absorbance at 517 nm. Standard ascorbic acid (1 mM) showed the maximum absorbance of 90.36 ± 1.05 µg/mL, and was taken as a reference solution in this DPPH antioxidant assay.

The formula used to calculate the percent (%) inhibition was

DPPH percentage (%) inhibition = [(X-Y)/X] x 100

Where
X = concentration of control &
Y = concentration of sample extract

2.5.2. FRAP assay

The FRAP assay was used to determine the free radical scavenging activity (RSA) or antioxidant activity of medicinal plant extracts [32]. Phosphate buffer (0.2 M) was prepared by taking 800 mL of water in 1,000 mL graduated flask and added with 8 g NaCl, 1.44 g disodium hydrogen phosphate, 0.2 g potassium chloride, 0.24 g potassium dihydrogen phosphate, and pH was adjusted to 6.6 using HCl and the volume was adjusted using deionized water. 1% potassium cyanide was prepared by mixing the potassium ferricyanide (1 g) in 100 mL of deionized water or dH_2O. 10% trichloroacetic acid stock solution was prepared by mixing trichloroacetic acid (10 g) in 100 mL of deionized or dH_2O. 0.1% ferric chloride stock was prepared by dissolving the ferric chloride (100 mg) in 100 mL of dH_2O. The standard ascorbic acid (0.1%) solution was prepared by mixing ascorbic acid (1 mg) in 1 mL of water. A colored compound was formed in the mixture, resulting from the reaction of antioxidants with ferric chloride, trichloroacetic acid, and potassium ferricyanide. The absorbance value of this solution was then measured at 700 nm by spectrophotometer.

The formula used to calculate the percentage (%) inhibition was

FRAP percentage (%) inhibition = [(X-Y)/X] x 100

Where
X = concentration of control &
Y = concentration of sample extract

2.5.3. ABTS assay

ABTS, a radical cation decolorization assay, was also used to investigate the free radical scavenging potential of plant samples [33]. 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) were used in this experiment, which when mixed and stored in the dark at room temperature for 12–16 h before use resulted in the formation of $ABTS^+$ cation radical by chemical reaction between them. The dilution of $ABTS^+$ solution was prepared with methanol to attain an absorbance value of 0.700 at 734 nm. Then, 3.995 mL of diluted $ABTS^+$ solution was added with 5 µL of plant extract and after 30 min incubation in dark from initial mixing, the absorbance was measured. Each assay was accompanied by running an appropriate solvent blank.

The aqueous plant extracts were mixed well using a stirrer. About 25 µL of these extracts were added to the dilution buffer and was vortexed thoroughly. The working solution was placed in freezer until next use. 10 µL of assay buffer was added to separate tubes as a negative control and 10 µL of samples or Trolox standard was also added to the given individual wells of the assay tubes. The solution was again added with 20 µL of sample or Trolox to all tubes with standards and samples of extracts. About 100 µL of the ABTS solution was added to each tube. The tubes were then placed on plate shaker at room temperature. The reaction was allowed to proceed for about 5 min. 50 µL of stop solution per tube was added to stop the reaction. The standard substance used in this experiment was Trolox. The absorbance of each sample was measured for the potential inhibition at a wavelength of 734 nm using UV spectrophotometer.

The formula used to calculate the percentage (%) inhibition was

$ABTS^+$ percentage (%) inhibition = [(X-Y)/X] x 100

Where
X = concentration of control &
Y = concentration of sample extract

2.6. Statistical analysis

Each experiment was repeated in triplicate and was measured as the mean value of three replicative trials as standard error mean (SEM) (mean value ± SEM). For each plant species in all three methods, the mean value and standard deviation (SD) and the SD variance (SDV) were calculated to check the % inhibition or % antioxidant activity.

3. Results and Discussion

3.1. Determination of TPC

The antioxidant activity of medicinal plants is usually because of the presence of phenolic and flavonoid contents in them. The antioxidant potential is primarily because of redox properties possessed by phenolic compounds found in the medicinal plants or other plants and fruits [34].

It was observed that the aqueous extract of *Murraya koenigi*i showed highest TPCs of 75.17 ± 1.53 GAE µg/mg followed by *Aegle marmelos* extract which showed the value of 52.03 ± 1.08 GAE µg/mg, and lowest TPC content was determined in *Laurus nobilis* which exhibited the TPC content of 29.24 ± 1.41 GAE µg/mg (Figure 1).

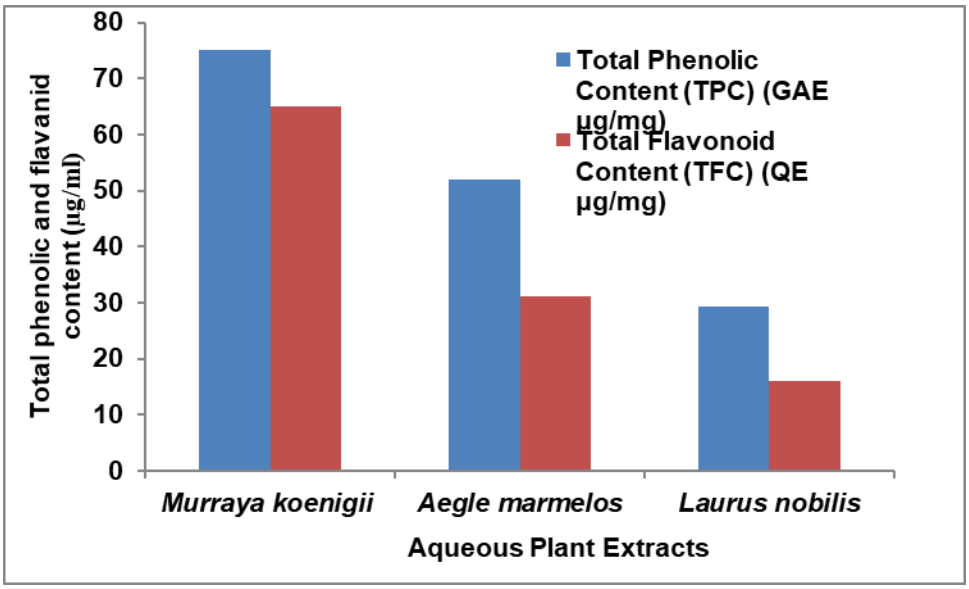

Figure 1 Total phenolic and flavonoid content in aqueous extracts of *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis*

3.2. Determination of TFC

Flavonoids are the various plant secondary metabolites like flavanols, flavones, and derivatives of tannins found excessively in plants with antioxidant potentials. The presence of free OH groups, especially 3-OH group is mainly responsible for the antioxidant activity of these flavonoids. Plant flavonoids can be used as potential antioxidant *in vitro* as well as *in vivo* [35].

It was observed that the *Murraya koenigii* showed highest TFCs, measured as 65.12 ± 1.18 QE µg/mg followed by *Aegle marmelos* extract which showed the value of 31.16 ± 1.18 QE µg/mg and lowest content of TFC was exhibited by *Laurus nobilis* which showed the values of 16.09 ± 1.21 QE µg/mg (Figure 1.)

3.3. *In-vitro* Antioxidant Activity

The aqueous extract of *Murraya koenigii* showed better DPPH radical scavenging activity with an IC50 value of 134 ± 1.9µg/ml as compared to the aqueous extract of *Aegle marmelos* which exhibited the DPPH scavanging activity with an IC50 value of 185 ± 0.21 µg/ml. Lowest DPPH scavanging activity was exhibited by *Laurus nobilis* with an IC50 value of 208 ± 0.57 µg/ml. Ascorbic acid as a standard antioxidant compound has showed the highest DPPH scavenging activity with an IC50 of 6.2±0.03 µg/ml (Figure 2).

Similarly, the extracts of *Murraya koenigii* showed higher ABTS radical scavenging activity with an IC50 value of 83±1.7 µg/ml as compared to that of *Aegle marmelos* which showed the value of 151±0.13 µg/ml. Lowest ABTS radical scavenging activity was observed in *Laurus nobilis* 182±0.24 µg/ml. Trolox as a standard antioxidant compound has showed the highest ABTS scavenging activity with an IC50 of 5.3 ± 0.02 µg/ml (Figure 2).

The ferric reducing capacities of aqueous extracts of all the three plants were tested. The standard L-ascorbic acid showed 85.48 ± 1.56% at 500 µg/mL concentration. The highest activity of 78.24 ± 1.21% was shown by *Murraya koenigii* aqueous extract, followed by *Aegle marmelos* which showed the reducing capacity of 69.72 ± 0.52% and lowest activity was exhibited by aqueous extract of *Laurus nobilis* 9.82 ± 0.48%. The concentration of all the aqueous extracts were 500 µg/mL (Figure 2).

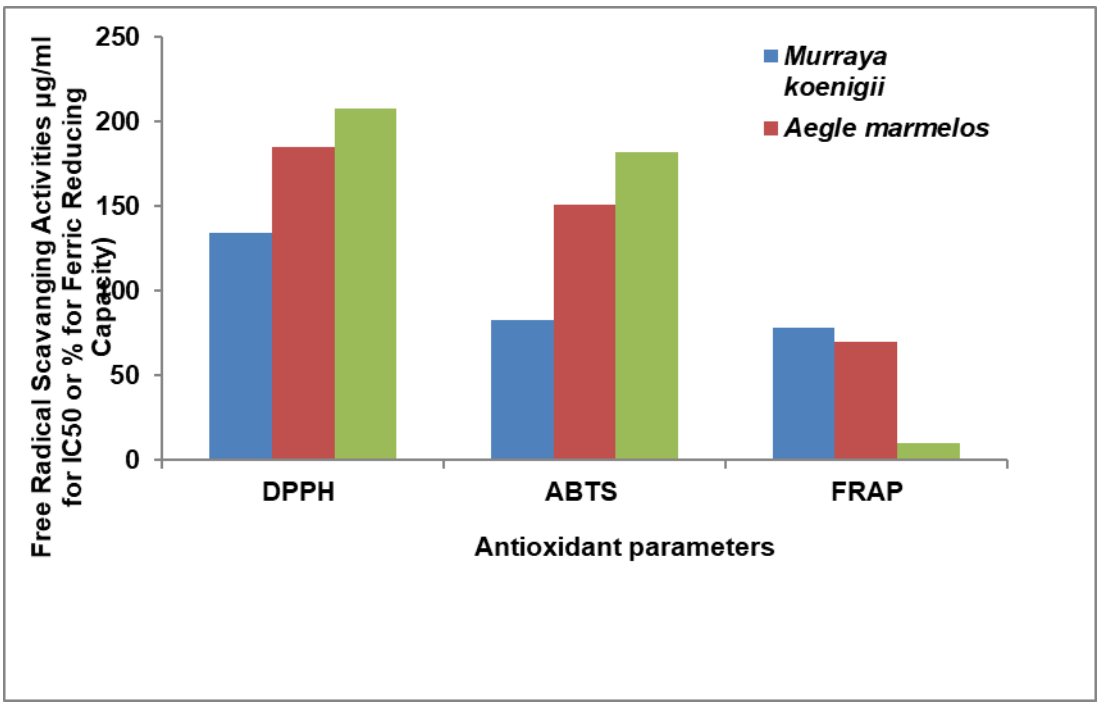

Figure 2 Free radical scavenging activity by DPPH and ABTS assay and Ferric reducing ability of aqueous extracts of *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis*

The antioxidant activity of the aqueous plant extracts of Murraya *koenigii, Aegle marmelos* and *Laurus nobilis* were evaluated using DPPH, FRAP and ABTS antioxidant assays. The order of antioxidant potential observed of the plants were *Murraya koenigii,> Aegle marmelos > Laurus nobilis* (Table 1). It was observed that the reductones present in these medicinal plants may be responsible for the reducing properties of the extracts and the reaction may be dependent for inhibiting the free radical chain by providing a hydrogen atom or may be by reaction with certain compounds of peroxides to avoid the peroxide development [36].

Table 1 IC50 VALUES (µG/ML) of aqueous extracts of *Murraya koenigii, Aegle marmelos* and *Laurus nobilis* in different antioxidant assay models

Extracts	**Antioxidant assay models**		
	DPPH	**ABTS**	**FRAP**
MURRAYA KOENIGII	134 ± 1.9	83 ± 1.7	78.24±1.21
AEGLE MARMELOS	185 ± 0.21	151 ± 0.13	69.72±0.52
LAURUS NOBILIS	208±0.57	182±0.24	9.82±0.48
L-ASCORBIC ACID	6.2± 0.03	-	85.48±1.56
TROLOX	-	5.3 ± 0.02	-

The antioxidant activity of the extracts were correlated with the levels of phenol and flavonoid compounds possessed by these medicinal plants. The free radical scavenging potential observed in these medicinal plant samples was because of the presence of some natural source such as phenol, flavonoid, or tannin contents. The free radical scavenging potential observed in these medicinal plant samples was because of the presence of some natural source such as phenol, flavonoid, or tannin contents. In this experiment, the free RSA of various medicinal plant samples was measured using

different extracts depending upon the ability to eliminate the free radicals using synthetic DPPH. The reactivity of different compounds with the stable free radicals was because of the odd number of electrons present in them. The total antioxidant potential of these medicinal plants was because of high amount of polyphenol and other phytochemical components found in them. These findings also indicated that all the tested medicinal plants samples are likely to possess significant levels of free RSA although comparatively less than standard ascorbic acid. So, this research suggested that all medicinal plants possess a significant antioxidant potential and can be efficiently applied as an important antioxidant source for the treatment and inhibition of widely spreading oxidative stress related degenerative diseases like cancer, cardiovascular and inflammatory joint disorders, atherosclerosis, dementia, diabetes, asthma, and eyes related degenerative diseases.

So, findings of our study are strongly supported by earlier studies and are correlated to above mentioned studies. The presence of phenolic and flavonoid contents in our studied plant extracts are responsible for antioxidant and scavenging effects to free radicals and effective to treat the diseases caused due to accumulation of ROS and free radicals in the body.

Different levels of scavenging activity were focused for all extracts in all used assays like DPPH, ABTS, and FRAP assays. It was also observed that the electron donating and/or free radical scavenging properties were responsible for the possible antioxidant potentials of these plant extracts which has been always concentration dependent. The phenol and flavonoid contents from these plants were observed as a potential source of natural antioxidants which can be efficiently used in the inhibition of oxidative stress associated diseases. This research may also lead to additional investigation of other specific compounds in various medicinal plants and their antioxidant potentials *in vivo* using various antioxidant assays. Thus, from these findings, it was concluded that the medicinal plants used for this study are important source of natural antioxidants like phenols, flavonoids, tannins etc., and can be efficiently used in the treatment of various oxidative stress related diseases, most importantly cardiovascular disorders and cancers.

3.4. Statistical Analysis

All data presented were analyzed in triplicate or quadruplet and mean values were presented in the table and text with respective standard deviations (SD).

4. Conclusion

We investigated the antioxidant efficacy of traditional medicinal plants like *Murraya koenigii* (curry leaf), *Aegle marmelos* (bael), and *Laurus nobilis* (bay leaf). Both the plants have shown significant antioxidant properties by scavenging DPPH radical, ABTS radical and reducing ferric ion. But greatest antioxidant properties were exhibited by *Murraya koenigii* leaves as compared to the pulp of *Aegle marmelos* and *Laurus nobilis*. The study of the *antioxidant* potential of *Murraya koenigii*, *Aegle marmelos* and *Laurus nobilis* reveals that these plants possess significant bioactive compounds that contribute to their antioxidative properties. Phytochemicals such as flavonoids, phenolic acids, alkaloids and essential oils present in these species play a crucial role in scavenging free radicals, thereby mitigating oxidative stress and associated cellular damage. *Murraya koenigii* exhibits potent antioxidant activity, attributed to its high content of phenolic compounds and flavonoids. Its extracts have been shown to effectively neutralize various reactive oxygen species (ROS) and protect biomolecules like DNA, proteins, and lipids from oxidative damage. A*egle marmelos*, known for its traditional use in Ayurvedic medicine, also demonstrates strong antioxidant capacity. The presence of bioactive constituents like tannins, flavonoids, and coumarins contributes to its ability to inhibit lipid peroxidation and stabilize free radicals, making it a promising candidate for the prevention of oxidative stress-related disorders. *Laurus nobilis*, commonly used as a culinary spice, contains essential oils rich in eugenol and other phenolic compounds. These components impart substantial antioxidant activity, which may offer protective effects against oxidative stress in biological systems.

Collectively, the findings support the potential of these plant extracts as natural antioxidants. Their integration into therapeutic applications or as dietary supplements could provide a natural means of combating oxidative stress, thus contributing to the prevention and management of various oxidative stress-related diseases. Further research involving clinical trials and mechanistic studies is recommended to fully elucidate their anti-oxidative mechanisms and therapeutic efficacy.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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