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EVALUATION OF ANTIOXIDANT PROPERTIES, TOTAL PHENOLIC AND FLAVONOID CONTENT OF SIX MEDICINAL PLANTS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The medicinal plants, *Aloe vera* (Kattarvazha), *Ocimum tenuiflourm* (Tulsi), *Azadirachta indica* (Neem), *Thymus vulgaris* (Thyme), *Piper betel* (Betel leaf), *Acalypha indica* (Kuppaimeni) were selected for management of stored grain pest management. The antioxidant activity, total phenolic and total flavonoid content of these six medicinal plant extracts were evaluated by using DPPH free radical, Folin-Ciocalteu and Aluminium chloride method, respectively. The total Phenolic content of the extracts was determined spectrophotometrically according to the Folin-Ciocalteu procedure and ranged from 127.0 mg/g to 339.6 mg/g on fresh leaves basis. The total flavanoid content of extracts were determined by aluminium chloride colorimetric assay and the values ranged from 0.16 mg/g to 4.00 mg/g of fresh weight. The results showed that the betel leaf extracts in acetone have highest (80.35%) DPPH Scavenging activity. The use of these plant extracts for the pest management in storage facilities may provide additional medicinal values to stored food commodities.

Keywords: Antioxidant activity; phenolic content; flavanoid content; phytochemical screening; GC-MS analysis.

1. INTRODUCTION

Medicinal plant is one of the most significant fields of traditional medicine around the world, and herbal remedies for a variety of medical conditions are becoming more popular. Phytochemical components found in plants are increasingly being related to their therapeutic potential [1]. Recent studies have revealed that natural antioxidants derived from medicinal plants protect against the toxic and hazardous effects

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of free radicals and have a wide range of pharmacological effects, including antimicrobial, antimutagenic, antiallergic, antioxidant free radical scavenging activity, and anticarcinogenic effects [2-4]. Antioxidants derived from medicinal or edible plants have recently attracted a lot of attention as potential treatment [5, 6]. According to ethnomedical literature, polyphenols are active substances found in plants which can be used to treat a variety of ailments.

Phenolic compounds are secondary plant metabolites characterized by the presence of one or more aromatic rings possessing at least one hydroxyl group attached to the aromatic structure [7]. The phenolic compounds have several bioactivities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [8-11] among others, and diets rich in phenolics are associated with human health benefits. Given the insects' herbivore feeding behavior and their complex interactions with plants, it would be expected that insects ingest plant phenolic compounds.

The phenolic and flavonoid compounds with antioxidant activity have raised interest in nutrition and food science [12]. Phenolic and flavonoid compounds are plant secondary metabolites which have an aromatic ring and at least one hydroxyl group [13]. Phenolic compounds are effective electron donors because their hydroxyl groups can directly contribute to antioxidant action [14].

Medicinal plants are widely used it as an alternative medicine source again for prevention and treatment of a variety of problems. Plant products' antioxidant properties are primarily due to phenolic substances such as flavonoids, phenolic acids, and tannins, as per recent study [15]. A rush of studies has based on the natural antioxidants in the search of getting sources of potentially safe, effective, and low-cost antioxidants [16]. Natural compounds containing free radical scavengers get a well therapeutic effect [17]. Plant phenolics are found abundantly in naturally, with flavonoids making up the majority half of them [18]. Antioxidant, antimutagenic, anti-carcinogenic, but now with the possibility to change gene expression, phenolics have a wide range of biochemical properties [19]. The phenolics, the largest group of phytochemicals, are responsible for the majority of antioxidant capacity in plants and plant products [20]. Flavonoids are the most abundant group of naturally occurring polyphenols, and they can be found in various plant sections in both free form and as glycosides. The flavones and flavonols are the most widely implemented phenolics, flavones, and flavonols [21]. It can scavenge harmful free radicals including such super oxide and hydroxyl radicals because to their polyphenolic composition [22]. The pest control board makes a large number of efforts to control the pests with plant-derived compounds. Therefore, researchers are developing new methods that can have minimal side effects on the environment and organisms. Their first attempt was to use the compounds obtained by aromatic plants to control the pests of stored goods [23].

Based on extensive evidence of biological effects of phenolic components, the study focused on measuring antioxidant, total phenolics, and flavonoids in six medicinal plants with different species. Six medicinal plants were explored in this research. viz., Kattarvazha, Tulsi, Neem, Thyme, Betel leaf and Kuppaimeni were screened for their antioxidant, total phenolics and flavonoid contents.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of six medicinal plants *Aloe vera* (Kattarvazha), *Ocimumtenuiflourm* (Tulsi), *Azadirachtaindica (*Neem), *Thymus vulgaris (*Thyme), *Piper betel (*Betel leaf), *Acalyphaindica (*Kuppaimeni) were collected from Thanjavur, Tamil Nadu, India (Table 1) and used for the study of antioxidant, total phenolics and flavonoid contents.

2.2 Extraction

The fresh leaves (50 g) were washed and ground in a mixer with an equal quantity of water (1:1). Similarly, the extract was prepared with acetone in the same quantity separately. They were kept overnight at 4°C and filtered using a muslin cloth followed by centrifugation (5000 rpm) and filtration (Whatman filter paper). The filtrate was collected in a 100 ml storage container and stored at 4°C in a refrigerator. Preliminary extract was subjected to get 100% stock solution. The extracts were used for antioxidant, total phenolic and flavonoid assay.

2.3 Antioxidant Activity of Six Medicinal Plants Extracts

The free radical scavenging ability was determined by using the stable radical DPPH. The crude extracts sample (100 µg/ml) were prepared using 0.3ml of the plant extract in a test tube added with 1ml of 0.3mM methanolic solution of DPPH. The solutions were mixed thoroughly followed by incubation at 30ºC for 20 min. The absorbance value of the sample was measured at 517nm in a UV spectrophotometer (Make: Shimadzu; Model: UV-1800). The percentage of DPPH radical scavenging activity was theoretically calculated by the following formulae [24].

DPPH radical scavenging activity (%) = [Reference OD – Sample OD] / Reference OD× 100.

2.4 Phytochemical Screening for Six Medicinal Plant Extracts

The plant extracts were screened for the presence of biologically active compounds like glycosides, tannins, quinones, flavonoids, terpenoids, coumarins, saponin and steroids under qualitative analysis as described by Chitravadivu *et al.* [25].

2.5 Total Phenolic Determination (TPC)

The total phenolic content of medicinal plant extracts was determined by using folin ciocalteu phenol reagent. The plant extracts were prepared in different concentrations (0.1, 0.2, 0.4, 0.6, 0.8,1.0ml) from stock solution. From each concentration, 0.3 ml of plant extracts was taken in test tubes and 0.5 ml of folin ciocalteu phenol reagent was added (FC reagent was dissolved in distilled water with 1:1 ratio). Then 1 ml of 20% sodium carbonate was added in each tube and finally, the mixture is mixed properly by using vortex and then the test tube were kept in a dark condition for 60 min. The absorbance spectra were recorded at 725 nm in a UV spectrophotometer ((Make: Shimadzu; Model:UV-1800) [26]. The experiments were performed in triplicate and the results were expressed in milligram of gallic acid equivalent (mg GAE).

2.6 Total Flavonoid Content (TFC)

The total flavonoids resent in the medicinal plant extract was estimated by a colorimetric method using aluminium chloride and sodium hydroxide. From stock solution, 1 ml of extract (1 mgml-1 of solvent) was added to 3 ml methanol, 0.1ml 10% aluminium chloride, 0.1ml 1M potassium acetate and 3.8 ml distilled water. The mixture was incubated for 30 min at ambient temperature and the absorbance was measured at 415 nm using UV spectrophotometer (Make: Shimadzu; Model:UV-1800).The total flavonoid content was measured from calibration curve using quercetin as standard and expressed in mg of quercetin equivalents/g dry weight [27]. The experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activities of Medicinal Plant Extracts

The DPPH has been widely used to evaluate the free radical scavenging activity of antioxidant. The free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method, it is possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm in UV Spectrometer (Make: Shimadzu; Model: UV-1800). The resulting methanolic solution (1.0 ml) of Neem, Tulsi, Thyme, Betel leaf, Kattarvazha and Kuppaimeni leaf extracts was taken for the experiment. The decolorization of DPPH by the effect of the extracts were measured at 517 nm. The results of DPPH radical scavenging assays of methanol (control) and plant extracts with different extracts (Aqueous and Acetone) showed free radical scavenging activity of the different plant extracts (Table 1) at a concentration of 300 µg /ml. The results showed that the neem extracts in acetone (80.35%) have higher DPPH Scavenging activity.

3.2 Phytochemical Screening Six Medicinal Plant Extracts

Phytochemical studies showed that glycosides, tannins, quinones, flavonoids, terpenoids, coumarins, saponin and steroids were found in thyme extracts (Table 2). All the plant extracts showed the presence of saponins and flavanoid. The presence of these phytochemical components is responsible for the observed the activity of the plant leaf extract. The medicinal plants extracts are being used traditionally for the treatment of inflammation, wound healing, antiseptics, cough, stomatitis and some fungal infection.

3.3 Total Phenolic Content of Medicinal Plant Extracts

The quantitative determination of total phenol was carried out using FolinCiocalteu reagent in terms of gallic acid equivalent. The term of phenolic refers to any compounds in a phenolic group which are high diversified group of phytochemicals derived from phenylalanine and tyrosine and biosynthesised in plants through the pathway. There is variation in total phenol content ranging from 127 mg/g to 339.6 mg/g (Table 3).The results indicated that the total phenolic content was found to be high in neem extracts (339.6 mg/g) followed by Kuppaimeni (256.6 mg/g).

3.4 Total Flavonoid Content of Medicinal Plant Extracts

The quantitative total flavonoid determination was performed by precipitating the extracts with aluminum chloride in an alkalinized medium. The

Table 1. DPPH scavenging activity of medicinal plant with different extracts

Plant extracts	DPPH Scavenging activity (%)	
	Aqueous extract	Acetone extracts
Kattarvazha	26.40 ± 11.30^{b}	24.98 ± 5.80^{b}
Tulsi	61.88 ± 7.55^{a}	52.60 ± 23.10^{ab}
Neem	49.41 ± 12.81^{ab}	68.93 ± 8.24^{a}
Thyme	42.46 ± 14.43^{ab}	50.80 ± 17.50^{ab}
Betel leaf	67.38 ± 2.96^{a}	80.35 ± 3.59^{a}
Kuppaimeni	46.06 ± 9.77^{ab}	74.33 ± 16.08^{a}

Values with different letters in the same column differ significantly (P <0.05)

Table 2. Phytochemical screening of medicinal plant extracts

Phytochemical Test	Kattarvaza	Tulsi	Neem	Thyme	Betel leaf	Kuppaimeni
Tannins	+	+	+	+	-	-
Flavonoid	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Glycosides	+	-	-	+	+	+
Quinones	-	+	-	+	-	+
Terpenoids	+	+	+	+	-	+
Steroids	+	-	+	+	+	+
Coumarins	+	-	+	+	+	+

Note: (+) indicates the presence of phytochemicals while (–) indicates the absence of phytochemicals

Table 3. Total phenolic and flavonoid content of different medicinal plants

Plants	Total phenolic content (mg GAE/g)	Total Flavonoid content (mg QE/g)
Kattarvazha	127.0 ± 0.09^{d}	0.16 ± 0.00^{e}
Tulsi	197.6 ± 0.22^{c}	3.09 ± 0.01^{b}
Neem	339.6 ± 0.83^{a}	4.00 ± 0.00^{a}
Thyme	197.6 ± 0.03^{c}	2.03 ± 0.00^{d}
Betel leaf	193.3 ± 0.18^{c}	1.51 ± 0.00^{d}
Kuppaimeni	256.6 ± 0.13^{b}	3.98 ± 0.01^{a}

Total phenolic / flavanoid content ± SD in all treatments; Values with different letters in the same column differ significantly (P <0.05)

flavonoid in the presence of aluminum chloride have an intense yellow fluorescence when observed UV spectrophotometer. Total flavonoid content was expressed as mg quercetin equivalent (QE) per gram dry extract weight.The total flavonoid content of the plants was analysed and the results indicated that the total flavonoid content was found to be high in neem extracts (4 mg/g) followed by Kuppaimeni (3.98 mg/g) and Tulsi (3.09 mg/g) (Table 3).

Extract anti-oxidant activity could not be explained just by their phenolic component; adequate characterization was also needed [28]. Only flavonoids with a certain structure, specifically the hydroxyl position in the molecule, can function as scavenging free radicals and proton donors [29,30]. In moreover, the extracts incorporate additional combinations of a variety of chemicals with various actions.

There is no connection between the total phenolic content of medicinal plant extracts and associated antioxidant activities. This could be due to the availability of additional phytochemicals such ascorbic acid, tocopherol, and pigments, that contribute to total antioxidant capacity. Additionally, the Folin-Ciocalteu method does not provide an absolute quantity of phenolic substances contained. Variable types of phenolic compounds have different antioxidant properties based on their structure. The extracts may contain a variety of phenolic chemicals with varying antioxidant properties. Polyphenols are phytochemical substances made up of phenylalanine and tyrophosphate [31]. The methanol extract used to have the highest combined phenolics concentration, whereas the contents of the residual aqueous fraction were significantly lower, which is similar to previous research [32]. Plant polyphenols are also useful because the scavenging properties on their hydroxyl groups. There are several types of phenolic compounds from plants, the most important of which are flavonoids, which have strong antioxidant properties [33]. Flavonoids are plant-derived antioxidants that are considered to be beneficial to

health. Studies have also shown that flavonoid derivatives have antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic capabilities [9, 34]. Singlet oxygen and other free radicals are one of the most oxidising compounds [35]. It has been related to a number of conditions. The results findings suggested that phenolic acids and flavonoids may be the major contributions to antioxidant activity, similar to the previous findings for various plant extracts [36].

Based on previous research, six medicinal plants were chosen for the current study among thousands of medicinal plants [37-43]. The antioxidant capacity of each plant's methanolic extract was analyzed using the DPPH radical scavenging assay and the ferric reduction assay because a single universal approach to evaluating plant antioxidant abilities cannot be depend upon. The DPPH free radical scavenging test is a popular method for evaluating antioxidant activity in plant extracts because it is inexpensive, reliable, and easy to use. The DPPH radical becomes a stable molecule in the presence of an antioxidant by gaining one extra electron or hydrogen atom from the antioxidant, and UV absorbance reduces, showing the scavenging activity of natural synthetic antioxidants [44]. Plant needs phenols to function properly. Plant species have a linear association between total phenol and antioxidant activity due to the scavenging ability of their phenolic hydroxyl groups [45].

The antioxidant action of medicinal plants is assumed to be due to their flavonoid concentration. Flavonoids are singlet oxygen quenchers and scavengers of oxidising species such as superoxide anion, hydroxyl radicals, and peroxy radicals [46]. Flavonoids are polyphenolic chemicals found in nature that are classified chemically as flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones. The pharmacological effects of offlavonoids were closely linked to their functionalgroup. In addition, various chemical components in the extract could cause some influence. Furthermore, flavonoids may protect cell structure in a variety of ways; one of the most efficient, according to multiple studies, is their capacity to increase glutathione levels, a potent antioxidant.

According to this study, antioxidant assays revealed that plant extracts with higher antioxidant activity and higher phenol and flavonoid content can be a substantial source of natural antioxidants, which might help prevent the development of oxidative stress and related conditions. Although scientific knowledge on the medicinal herbs used in this study is minimal, a few of them showed high antioxidant activity.

4. CONCLUSION

The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. In the present study analysis of free radical scavenging activity and total phenolic and flavonoid content showed that mainly the medicinal plant extracts can be the potent source of natural antioxidants. The usage of these plant extracts during storage of grains and pest management may provide health benefits.

The present study reported the antioxidant activity, total phenolic and flavonoid contents of six medicinal plants. In order to realize the health benefits from potential plant sources, it is important to measure the anti-oxidant activity using various radicals and oxidation systems and Neem and kuppaimeni were found to possess the highest phenolic content while kuppaimeni acetone extract and betel leaf aqueous extract showed the highest antioxidant activity, thus attributing to their use as medical plants. Further, the biomolecules present in the extract which are active against these microbes needs to be characterized. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance to conventional synthetic antibiotics. Hence, this study holds importance in using medicinal plants as an alternative source for the management of storage of grains. Additional information on the dietary intake of medicinal plants selected in this study, and enhancing their bioavailability after various processing operations need to be elucidated in future. The study revealed the phenolic and flavonoid spectrum of medicinally important plants. Since these plants possess insecticidal activity to stored product pest consecutively, it can be regarded as promising plant species of natural plant sources for pest management of stored product commodities.

DISCLAIMER

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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