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Studies on the Phytochemicals and Antioxidant Activities from Medicinal Plants by Various Methods

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ABSTRACT

Antioxidant plays an important role in inhibiting and scavenging free radicals, thus, providing protection to human against infection and degenerative diseases. Now the modern research is directed towards Natural antioxidants from the herbal plants due to safe therapeutic values. In the present investigation, the Antioxidant activity of extracts from medicinal plants such as *Acalypha indica*, *Azadirachta indica*, *Euphorbia hirta*, *Ficus religiosa*, *Pongamia pinnata* and *Thespesia populnea* are evaluated for its free radical scavenging activity by adopting various *in vitro* methods. The extracts were investigated for the antioxidant activity using reducing capacity, Hydroxyl group reducing activity and DPPH methods and the phytochemicals contents such as alkaloids, carbohydrate, flavonoids, glycosides, phenolic contents, proteins, reducing sugars, saponins, steroids, tannins, and terpenoids, were analyzed qualitatively.

INTRODUCTION

Many herbal plants contains antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl, radicals, hydroxyl radicals. The concept of oxidative stress is that, when a balance between ROS production and antioxidant defenses is lost, 'oxidative stress' result which through a series of events deregulate the cellular function and leads to various diseases such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurodegenerative disease. Antioxidants are substances that neutralize free radicals and their actions.

Oxidative stress has been recognized to have a pathological role in many types of chronic diseases such as diabetes, heart disease and cancer. Oxidative stress occurs when the formation of free radicals increases (Elmastas *et al*., 2006). In oxidative stress, the balance between the formation of reactive oxygen species and amount of antioxidants is destroyed. Oxidative stress causes damage to cell components, such as proteins, lipids and nucleic acids (Rahimi *et al*., 2005; Wright *et al*., 2006; Gladine *et al*., 2007) and eventually leads to cell death (NazIroglu *et al*., 2004; Emekli-Alturfan *et al*., 2009). Antioxidant effects of various medicinal plants used in traditional therapeutics are associated with their antioxidant properties (Sathishsekar and Subramanian, 2005; Aiyegoro and Okoh, 2009).

Current scientific articles have focused on plant natural antioxidants (Emekli-Alturfan *et al*., 2009). Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins and phenolic acids (Lee *et al*., 2004; Horax *et al*., 2005). There are various methods to estimate the antioxidant activity of compounds in plant extracts and one method alone is unable to recognize all possible mechanisms characterizing an antioxidant (Dorman *et al*., 2003; Erkan *et al*., 2008).

MATERIALS AND METHODS

Preparation of plant extracts

Methanolic extract

The methanolic extract of *Acalypha indica, Azadirachta indica, Euphorbia hirta, Ficus religiosa, Pongamia pinnata* and *Thespesia populnea* plant was prepared based on a method previously

described by Virdi *et al*. (2003). A total of 500 g plant extract was weighed and mixed with 5 L (1:10) of methanol. The mixture was then, left for 1 h in the dark at 50°C. The mixture was then, filtered and evaporated to dryness under reduced pressure using a rotary evaporator to produce the yield. The extract was then kept at -80°C until use.

Antioxidant activity

Reducing power assay

In this case 0.5 ml of different fraction (l mg/ml) was added to 3ml Potassium ferrocyanide (1mM) solution. The mixture was shaken thoroughly and incubated for 10 minutes at room temperature. Finally, the mixture was tested spectrophotometrically at 700 nm using an appropriate blank of 3.5 ml of potassium ferrocyanide solution after every 10 minutes interval up to 30 minutes.

Hydrogen Peroxide

A solution of hydrogen peroxide (40 mmol/ l) was prepared in phosphate buffer (50 mmol/l, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 700 nm using a spectrophotometer. Plant extract (1 mg/ml) in distilled water was added to hydrogen peroxide and absorbance at 700 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

DPPH:

Ethanol solution of DPPH 0.05 mm (300 µl) was added to 4 ml of extract solution with different concentration. DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol 96% was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared.

Analysis of Phytochemicals (Harborne, 1998)

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996). Tests are alkaloids, carbohydrate,

flavonoids, glycosides, phenolic components, proteins, reducing sugars, saponins, steroids, tannins and terpenoids.

RESULTS AND DISCUSSION

Plants were identified as useful sources of natural antioxidants that can protect against oxidative stress and therefore, have a main role to protect against injuries from lipid peroxidation (Repetto and Llesuy, 2002). *Momordica charantia* (MC) contains strong antioxidant and free radical scavenging activities, which can be extracted from compounds such as flavonoids and phenols (Wu and Ng, 2008). The ferric thiocyanate (FTC) results showed that methanolic extract had greater antioxidant activity when compared with chloroform extract. Previous studies have claimed that the aqueous extract of herbal plants has high antioxidant activity against lipid peroxidation. The reaction in the FTC method is due to the MDA compounds from the linoleic acid oxidation in which, peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment (Al-Naqeeb *et al*., 2009).

Peroxides are slowly decayed to lower molecular compounds during the oxidation course (Behbahani *et al*., 2007). Methanol has stronger polarity than the chloroform solvent (Rahmat *et al*., 2003). The higher antioxidant activity found from the ferric thiocyanate method indicated that the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage. Thus, these data suggest that methanolic extract has a better beneficial effect against lipid peroxidation when compared with chloroform extract. The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts (Elmastas *et al*., 2007). Ansari *et al*. (2005) reported that a heated methanol, water extract of MC showed higher free radical antioxidant activities than a cold extract of *Momordica charantia*. High free radical antioxidant activity of wild fruit (Wu and Ng, 2008) and *Momordica dioica Roxb*. Leaves have been found in *Momordica charantia* ethanolic extracts (Jain *et al*., 2008; Santhi and Sengottuvel, 2016 and Sherikar and Mahanthesh, 2015). Therefore, these results suggest that the difference in radical scavenging of these two extracts may be due to differential solubility of the MC compounds in the solvents. We concluded that the methanolic extract (polar extract) with maximal inhibition of free radicals is a more potent extract when compared with the chloroform extract (non-polar).

In the present investigation suggest that the antioxidant activity of some medicinal plants with methanolic and ethanolic extracts of *Acalypha indica, Azadirachta indica, Euphorbia hirta, Ficus religiosa, Pongamia pinnata* and *Thespesia populnea* were determined with various methods of hydrogen peroxide, reducing power assay and DPPH methods followed with absorbance at 700 nm was used for optical density. According to the hydrogen peroxide 0.2±0.006,0.38±0.011, 0.08±0.002, 0.21±0.006, 0.14±0.004 and 0.13±0.003% with *Acalypha indica, Azadirachta indica, Euphorbia hirta, Ficus religiosa, Pongamia pinnata* and *Thespesia populnea* plant extract was introduced whereas reducing power assay also absorbed with excellent activity *Azadirachta indica* and followed by *Ficus religiosa* plant extract and other plants.

In the case of DPPH maximum percentage was 1.7±0.05, 1.54±0.04, 1.3±0.04% activity observed with *Acalypha indica, Azadirachta indica, Euphorbia hirta, Ficus religiosa, Pongamia pinnata* and *Thespesia populnea* plant extract respectively.

In the present study that the some of the phytochemical contents were analysed such as alkaloids, carbohydrate, flavonoids, glycosides, phenolic contents, proteins, reducing sugars, saponins, steroids, tannins and terpenoids were analyzed qualitatively. The medicinal plant extract were found in various levels of antioxidant activity in the three above systems. Moreover from the result showed that the methanolic extract had high antioxidant potential and these plants could provide a chemical basis for future in the society.

Table 1: Determination of antioxidant activity of some medicinal plants by various methods

Sr. No.	Name of the medicinal plants	Hydrogen peroxide (%)	Reducing power assay (%)	DPPH (%)
1	*Acalypha indica*	0.21±0.006	1.5±0.045	1.7±0.05
2	*Azadirachta indica*	0.38±0.011	1.09±0.032	0.9±0.02
3	*Euphorbia hirta*	0.08±0.002	0.52±0.015	0.93±0.03
4	*Ficus religiosa*	0.21±0.006	1.4±0.042	1.54±0.04
5	*Pongamia pinnata*	0.14±0.004	0.7±0.021	0.7±0.02
6	*Thespesia populnea*	0.13±0.003	00.9±0.027	1.3±0.04

Table 2: Qualification phytochemical analysis some medicinal plants extract

Sr. No.	Name of the test	*P. pinnata*	*T. populnata*	*A. indica*	*E. hirta*	*F. religiosa*	*A. indica*
1	Alkaloids	+	+	+	+	-	+
2	Carbohydrates	+	-	-	-	-	+
3	Flavonoids	+	+	+	-	+	+
4	Glycosides	+	+	-	+	-	+
5	Phenolic component	+	-	+	-	+	-
6	Proteins	+	+	-	-	-	+
7	Reducing sugar	+	-	+	-	+	+
8	Saponin	+	-	+	+	-	+
9	Steroids	+	+	+	+	-	+
10	Tannins	+	-	-	+	+	+
11	Terpenoids	+	+	+	-	+	-

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