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Saima Tauseef
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Gulshan campus, Karachi

Muhammad Shaiq Ali
H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi

Aqeel Ahmed
Department of Microbiology, University of Karachi.

Muhammad Imran Ali
H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi

Zeeshan Ahmed
H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi

Sikander Khan Sherwani
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Gulshan campus, Karachi

Ghafoor Ahmed
H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi

Patricia A. Onocha
Department of Chemistry, University of Ibadan, Nigeria

Ngoupayo Joseph
University of Yaounde I, Cameroon

Alain Francois Kamdem Waffo
Department of Chemistry, Faculty of Science, University of Douala, Cameroon

Farah Tauseef
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Gulshan campus, Karachi

Correspondence
Saima Tauseef
Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Gulshan campus, Karachi
Email: saimafaraz@yahoo.com

In vitro Antioxidant activity analysis of five medicinally important plants

Saima Tauseef, Muhammad Shaiq Ali, Aqeel Ahmed, Muhammad Imran Ali, Zeeshan Ahmed, Sikander Khan Sherwani, Ghafoor Ahmed, Patricia A. Onocha, Ngoupayo Joseph, Alain Francois Kamdem Waffo, Farah Tauseef

ABSTRACT

Reactive oxygen species are the cause of different ailments such as cancer. The intake of foods rich in antioxidant compounds may prevent the occurrence of these diseases. Thus, antioxidants play important role in controlling the free radical disorders. In the present work, the antioxidant potential of 48 different extracts, fractions and pure compounds of five medicinally important plants was evaluated by using the DPPH method. Methyl gallate obtained from the *Acalypha torta* showed good antioxidant activity (EC_{50} = 2.3 µg/ml). Vanillic acid isolated from *Clerodendrum formicarum* showed moderate antioxidant activity (EC_{50} = 50 µg/ml). *Acalypha segetalis* and *Erythrina* were found inactive during the study (EC_{50} >200 µg/ml). The above plants could provide rich supply of natural antioxidants and may possible preventive agents in ROS related ailments.

Keywords: Antioxidant activity, DPPH, Reactive oxygen species (ROS)

1. Introduction

Since cancer is the second leading cause of deaths in the human being, therefore scientists are interested in searching the different causes of this ailment. The important cause of cancer is the formation of reactive oxygen species (ROS)[1]. Different biochemical and physiological reactions in the human body produces ROS which have ability to target the DNA and bringing change in DNA, in most cases, it results in cancer[2].

Cancer is abnormal growth of cells in human body which affects and damages the normal function of the body and causes death. The antioxidants are the compounds which play important role in inhibition of production of free radicals in the human body. They not only decrease the damage of DNA and also inhibit the abnormal growth of cells in the human body. A huge variety of antioxidants are present in plants such as polyphenols, vitamin E, vitamin C, β-carotene, Selenium, *etc*., that prevent free radicals formation in human body[3].

Several phytochemicals of plants, vegetables and fruits are known to possess antioxidant potential. The intake of plants, vegetables and fruits in daily life may help to reduce the risk of ROS mediated diseases such as cancer in human[4]. Plants contain more than 8000 polyphenolic compounds[5] which possess antioxidant and anticancer activities[6]. In present research, therefore, we determined the antioxidant activities of different extracts/ and pure compounds of five medicinal plants.

2. Material and Methods

2.1 Extraction Procedures from Medicinal Plants

2.1.a *Acalypha segetalis*

Leaves of *Acalypha segetalis* were collected form Sabo, Ibadan, Oyo State (Niregia) in June 2004 and identified by Mr. Felix Usang of the Forest Research Institute (FRIN), Ibadan, Nigeria where voucher specimen is deposited (Herbarium # FHI107321).The dried leaves of *Acalypha segetalis* (dried weight 4.2 kg) were soaked in methanol (6.5 L) for a period of eight days. The percolation procedure was repeated three times. Methanol was evaporated at low temperature to avoid thermal decomposition of the natural products.

The combined crude methanolic extract (105 gm) was subjected to silica gel column chromatography and the column was eluted with hexane, hexane - ethyl acetate, ethyl acetate, ethyl acetate-methanol and finally, methanol with few drops of acetic acid as mobile phase. Several constituents were obtained and characterized.

2.1.b *Acalypha torta*

Leaves of *Acalypha torta* were collected form flower kingdom in Ibada, Oyo State (Nigeria) in June 2004 and identified by Mr. Felix Usang of the Forest Research Institute (FRIN), Ibadan, Nigeria where voucher specimen is deposited (Herbarium # FHI107324). The dried leaves of *Acalypha torta* (dried weight 3.8 kg) were soaked in methanol (8.0 L) for a period of ten days. The percolation procedure was repeated three times. Methanol was evaporated at low temperature to avoid thermal decomposition of the natural products. The combined crude methanolic extract (60.0 gm) was subjected to silica gel column chromatography and the column was eluted with hexane, hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and finally, pure methanol with few drops of acetic acid as mobile phase. Several constituents were obtained and characterized.

2.1.c *Aegle marmelos* Corr.

Fresh unripe fruits of *Aegle marmelos* (20 kg) were collected from nearby the Karachi University campus and identified by Prof. Usmanghani Khan, Faculty of Eastern Medicines, Hamdard University, Karachi, where a voucher specimen is deposited (# UK-63).The collected fruits were chopped into small pieces and soaked in ethanol (27 L) for five days. Ethanol was evaporated under reduced pressure on a rotary evaporator to avoid thermal decomposition. The gummy material thus obtained (902 gm) was suspended in water and the organic material was recovered in ethyl acetate. Again, ethyl acetate soluble part was concentrated *via* rotary evaporator (297 gm) and subjected to silica gel column chromatography using hexane, hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and finally, pure methanol as mobile phase. Several constituents were obtained and characterized[7, 8, 9].

2.1.d *Clerodendrum formicarum*

The leaves of *Clerodendrum formicarum* were collected in June, 2008, from Obili-Yaounde (Cameroon) and identified by Mr. Nana Victor of National Herbarium, Yaounde, Cameroon, where a voucher specimen is deposited (Herbarium # HNC-13658). The collected leaves were dried under shade for a period of seven days. The dried and powered material (6.0 kg) was then soaked in ethanol (12 L) for six days. The resulted extract was concentrated by means of evaporation under vacuum distillation (84.5 gm) to avoid thermal decomposition of natural products and subjected to silica gel column chromatography using hexane, hexane: ethyl acetate, ethyl acetate, and finally, ethyl acetate: methanol as mobile phase. Several constituents were obtained and characterized[21, 22, 23].

2.1.e *Erythrina sigmoidea*

The stem bark of *Erythrina sigmoidea* was collected by Dr. P.A. Onocha, Chemistry Department, University of Ibadan (Nigeria) in June, 2005 from Iseyin, Ibadan, Oyo State (Nigeria) and identified by Mr. Felix Usang of the Forest Research Institute, Nigeria (FRIN), Ibadan (Nigeria) where a voucher specimen (FHI-107098) is deposited.

The dried stem bark (4.75 kg) was percolated with methanol (8.0 L) at room temperature and the obtained extract was condensed by means of evaporation under low pressure (263 g). The obtained gummy mass then subjected to silica gel column chromatography using hexane, hexane: ethyl acetate and pure ethyl acetate as mobile phase. Several constituents were obtained and characterized[24, 25].

2.1.f *Erythrina vogelii*

The leaves of *Erythrina vogelii* were collected in February 2005 from Ngaoundere (Cameroon) and identified by Mr. Nana Laurent of National Herbarium, Yaounde (Cameroon), where a voucher specimen is deposited (SRF-20693).

The shade-dried leaves (8.0 kg) were extracted with ethanol (13.5 L) at room temperature and the resulting extract was condensed under vacuum. The obtained residue (190 g) was subjected to silica gel (3 kg) column chromatography. Hexane, hexane: ethyl acetate, ethyl acetate were used as mobile phase several constituents were obtained and characterized

2.2 Antioxidant activity Procedure

In this study, antioxidant activity of the test sample was determined by using the DPPH method as described by Lee and coworkers in 1998[10]. Solution of DPPH was prepared in ethanol (333 µM). Briefly, 10 µL of test sample and 90 µL solution of DPPH (final concentration of test sample was 200 µg/ mL and 300 µM of DPPH) was added in 96-well microtiter plates and incubated at 37 ºC for 30 minutes. Absorbance was measured at 515 nm by using a spectrophotometer. Percent inhibition of radicals by treatment of test sample was determined by comparison with a DMSO treated control group.

$$\% \text{ Inhibition} = \frac{(\text{absorbance of the control} - \text{absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

Ascorbic acid was used as control. The EC_{50} values calculated denotes the concentration (in µg/ml) of sample required to scavenge 50% of DPPH free radicals.

Table 1: Antioxidant Activity of extracts/fractions and pure compounds

S.no	Plant Part Used	Crude Fractions / Compounds	Crude Fraction/ Class Of Compound	%Inhibition (At 200 µg/Ml)	EC_{50}values (µg/ml)
1.	Leaves of *Acalypha segetalis*	1.5 % methanol in ethyl acetate extract	Crude	-	>200
2.	Leaves of *Acalypha segetalis*	2 % methanol in ethyl acetate extract	Crude	15	>200
3.	Leaves of *Acalypha segetalis*	β-sitosterol	Sterol	2	>200
4.	Leaves of *Acalypha segetalis*	β-sitosterol-glucoside	Sterol	9	>200
5.	Leaves of *Acalypha torta*	Drops methanol in ethyl acetate extract	Crude	8	>200
6.	Leaves of *Acalypha torta*	1.0 % methanol in ethyl acetate extract	Crude	16	>200
7.	Leaves of *Acalypha torta*	2.5 % methanol in ethyl acetate extract	Crude	8	>200
8.	Leaves of *Acalypha torta*	1.0 % methanol in ethyl acetate extract	Crude	-	>200
9.	Leaves of *Acalypha torta*	Methyl gallate	Aromatic acid	76	2.3
10.	fruits of *Aegle marmelos*	8 % methanol in ethyl acetate	Crude	64	12.5
11.	fruits of *Aegle marmelos*	2.0 % methanol in ethyl acetate extract	Crude	-	>200
12.	fruits of *Aegle marmelos*	1.0 % methanol in ethyl acetate	Crude	-	>200
13.	fruits of *Aegle marmelos*	Kaempferol	Flavone	-	>200
14.	fruits of *Aegle marmelos*	phytol	Diterpene	2	>200
15.	Leaves of *Clerodendrum formicarum*	100 % ethyl acetate	crude	-	>200
16.	Leaves of *Clerodendrum formicarum*	0.5% ethyl acetate in hexane	crude	-	>200
17.	Leaves of *Clerodendrum formicarum*	Drops of methanol in ethyl acetate	crude	31	>200
18.	Leaves of *Clerodendrum formicarum*	0.5% methanol in ethyl acetate	crude	-	>200
19.	Leaves of *Clerodendrum formicarum*	1.0 % methanol in ethyl acetate	crude	-	>200
20.	Leaves of *Clerodendrum formicarum*	5% ethyl acetate in hexane	crude	19	>200
21.	Leaves of *Clerodendrum formicarum*	8% ethyl acetate in hexane	crude	11	>200
22.	Leaves of *Clerodendrum formicarum*	5% ethyl acetate in hexane	crude	-	>200
23.	Leaves of *Clerodendrum formicarum*	Drops of ethyl acetate in hexane	crude	70	50
24.	Leaves of *Clerodendrum formicarum*	15% ethyl acetate in hexane	crude	7	>200
25.	Leaves of *Clerodendrum formicarum*	100% chloroform	crude	5	>200

26.	Leaves of *Clerodendrum formicarum*	25% ethyl acetate in hexane	crude	-	>200
27.	Leaves of *Clerodendrum formicarum*	Betulin	triterpene	5	>200
28.	Leaves of *Clerodendrum formicarum*	Maslinic acid	triterpene	3	>200
29.	Leaves of *Clerodendrum formicarum*	Betulinic acid	triterpene	31	>200
30.	Leaves of *Clerodendrum formicarum*	Friedelin	triterpene	-	>200
31.	Leaves of *Clerodendrum formicarum*	Friedlan-3-ol	triterpene	13	>200
32.	Leaves of *Clerodendrum formicarum*	Vanillic acid	Aromatic acid	73	50
33.	Leaves of *Clerodendrum formicarum*	Formic acid A	Aromatic acid	14	>200
34.	Leaves of *Clerodendrum formicarum*	Formic acid B	Aromatic acid	-	>200
35.	Leaves of *Clerodendrum formicarum*	Vanilline	Aromatic acid	10	>200
36.	Stem Bark of *Erythrina sigmoidea*	1.0 % methanol in ethyl acetate	crude	-	>200
37.	Stem Bark of *Erythrina sigmoidea*	1.0 % methanol in ethyl acetate	crude	67	37.5
38.	Stem Bark of *Erythrina sigmoidea*	2.0 % methanol in ethyl acetate	crude	-	>200
39.	Stem Bark of *Erythrina sigmoidea*	Erythrinasinate	Cinnamic acid derivative	-	>200
40.	Stem Bark of *Erythrina sigmoidea*	Lupeol	triterpene	-	>200
41.	Leaves of *Erythrina vogelii*	1.0 % methanol in ethyl acetate	crude	46	>200
42.	Leaves of *Erythrina vogelii*	100 % ethyl acetate	crude	11	>200
43.	Leaves of *Erythrina vogelii*	1.0 % methanol in ethyl acetate	crude	14	>200
44.	Leaves of *Erythrina vogelii*	Drops of methanol in ethyl acetate	crude	7	>200
45.	Leaves of *Erythrina vogelii*	1.0 % methanol in ethyl acetate	crude	8	>200
46.	Leaves of *Erythrina vogelii*	Stigmasterol	sterol	-	>200
47.	Leaves of *Erythrina vogelii*	Benzaldehyde	Aromatic aldehyde	42	>200
48.	Leaves of *Erythrina vogelii*	Soyasapogenol-B	triterpene	6	>200
49.	Vitamin C	*	*	87	9.4

Key: *=not applicable, - = no antioxidant activity found

3. Structures

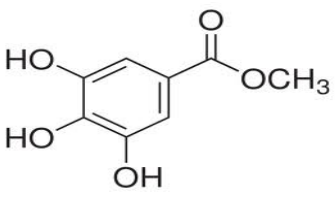

Fig I: Methyl gallate

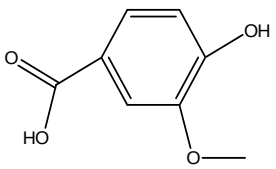

Fig II: Vanilic acid

4. Discussion

The antioxidant potential of 48 different extracts, fractions and pure compounds of five medicinally important plants (Table-1) was evaluated by DPPH free radical scavenging assay[10]. DPPH is a stable free radical; it is a commonly used substrate for the fast evaluation of antioxidant activity. An antioxidant can reduce the purple solution of DPPH free radicals into yellow. This color change is measured quantitatively at 515 nm by spectrophotometric absorbance[11].

Acalypha torta is used for the treatment of neonatal jaundice in the traditional system of medicinal treatment[12]. In the current work, methyl gallate (Figure: I) obtained from the plant showed better antioxidant activity (EC_{50} = 2.3 µg/ml) than vitamin C (Table-1). The vitamins C and E deficiency increases oxidation stress in the neonate which results in increases lysis of RBC and causes jaundice[13]. Thus our work, therefore, supports the use of this plant for traditional medicinal system.

Erythrina sigmoidea is traditionally employed for the cure of female sterility and peptic ulcer[14]. Oxidative stress may cause peptic ulcer[15] and female sterility[16]. Therefore, we studied antioxidant activity of this plant. In our results, 1.0 % methanol in ethyl acetate extract of stem bark of *Erythrina sigmoidea* showed moderate antioxidant activity (EC_{50} =37.5 µg/ml). *Acalypha segetalis* and *Erythrina* were found inactive during the study (Table-1). The inactivity of these plants may be the result of difference of time, weather and climate during sample collection which may alter the concentration of antioxidants in plants[17].

Aegle marmelos Linn. is native to India and is used in folk medicines[18]. In this study, 8% methanol in ethyl acetate extract of Aegle *marmelos* showed good antioxidant activity (EC_{50} =12.5 µg/ml) (Table-1). The antioxidant activity of leaf extract may be the result of phytochemicals such as tannins, alkaloids, flavonoids, phlobotannins, sterols, and flavonoid glycosides normally present in this plant[19].

Clerodendrum species have been used as antioxidant agents in various indigenous systems of medicines[20]. During this study, Ethyl acetate and hexane extract of leaves of *Clerodendrum formicarum and* Vanillic acid (a flavonoid) (Figure: II) isolated from this plant showed moderate antioxidant activity (EC_{50} = 50 µg/ml) (Table-1). This work is in accordance with previous report that the major group of compounds present in *Clerodendron* species is flavonoid and responsible for many biological activities of this plant[20]

5. Conclusion

The above compounds could be included in the list of natural antioxidants and may be used for the prevention of ROS related diseases such as cancer. However, a detailed study should be performed for their possible use in pharmaceutical industries.

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