Turk J Biol
35 (2011) 233-242

doi:10.3906/biy-0906-64

The antioxidant and DNA protection potential of Indian tribal medicinal plants

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Received: 06.07.2009

Abstract: Prevention and treatment of various degenerative diseases using traditional medicines is increasingly generating interest, especially in geriatric clinical research. This study aimed to evaluate the free-radical scavenging properties and potential to prevent DNA damage (from oxidative stress) of 56 extracts (polar and non-polar) from 14 medicinal plants (from 12 families) used by diverse Indian tribes and ethnic groups. Although widely known for traditional uses, these plants have not been extensively used for clinical purposes. Scavenging of DPPH$^{\bullet}$ radical by all the extracts was evaluated to estimate their antioxidant potential and rank them accordingly. Extracts occupying the top 10 ranks were further evaluated and extensively screened for their antioxidant potential by estimation of their ABTS$^{\bullet+}$-scavenging and Fe^{+3}-reducing properties. A cumulative ranking system was designed for these 10 plant extracts based on the results of the 3 antioxidant assays. Simultaneously, protection of DNA against photolyzed H_2O_2-induced oxidative damage by all 56 extracts was studied in pBR322. Considerable anti-genotoxic potential was exhibited by the top 3 extracts on the cumulative rank table, thereby proving the results of all the experiments were congruent. This is a preliminary report, which may yield a roadmap for isolating the active components from these extracts and designing geriatric medicines.

Key words: Tribal medicinal plants, antioxidant, oxidative damage, DNA protection, DPPH, ABTS

Hint kabilelerinin tıbbi bitkilerin DNA koruma ve antioksidan potansiyeli

Özet: Geleneksel ilaçların kullanımı ile çeşitli dejeneratif hastalıkların tedavisi ve önlenmesi özellikle geriatrik klinik araştırmalarında artan bir biçimde ilgi oluşturmaktadır. Bu çalışma çeşitli Hindistan kabile ve etnik grupları tarafından kullanılan 14 tıbbi bitkiye ait (12 familyadan) 56 özütün (polar ve polar olmayan) serbest radikal arındırıcı özellikleri ve DNA zararını engelleme potansiyelini değerlendirmeyi amaçlamıştır. Geleneksel kullanımları geniş çapta bilinmesine rağmen, bu bitkiler klinik amaçlar için büyük ölçüde kullanılmamaktadır. Tüm özütler ile DPPH radikalinin arındırılması onların antioksidan potansiyelini tahmin etmek ve sıralamak için değerlendirilmiştir. Ilk 10 sırada bulunan özütler Fe^{+3}-indirgeyici ve onların ABTS arındırıcı özelliklerinin tahmin edilmesiyle antioksidan potansiyelleri için geniş ölçüde izlenmiş ve daha dazla değerlendirilmiştir. Kümülatif bir sıralama sistemi 3 andioksidan temelli sonuçlara dayalı bu 10 bitki özütü için tasarlanmıştır. Aynı zamanda, 56 özütün tümüyle fotolize H_2O_2 indükleyicisinin oksidatif zararına karşı DNA'nın korunması pBR322'de çalışılmıştır. Dikkate değer anti-genotoksik potansiyel kümülatif sıra tablosunda ilk 3 özüt ile sergilenmiştir, dolayısıyla tüm deneylerden elde edilen sonuçlar uyumludur. Bu, geriatrik ilaç tasarımı ve özütlerden aktif bileşiklerin izolasyonu için bir yol haritası verebilen öncü bir ön çalışmasıdır.

Anahtar sözcükler: Kabilesel tıbbi bitkiler, antioksidan, oksidatif hasar, DNA koruma, DPPH, ABTS

Introduction

Molecules containing unpaired electrons are known as free radicals that cause tissue collapse by means of DNA, protein, and lipid damage (1). Free radicals, such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, are known as reactive oxygen species (ROS) (2). ROS, which are generated by normal physiological processes and various exogenous factors, cause oxidative stress, and thereby initiate peroxidation of membrane lipids, causing damage to a wide range of other biological molecules through a process that is thought to be implicated in the etiology of several diseases, including coronary artery diseases, stroke, rheumatoid arthritis, diabetes, and cancer (3,4). Such oxidative stress is also involved in the pathophysiology of aging and various age-related ailments, including cataracts, atherosclerosis, diabetes, Alzheimer's disease, etc. (5). The extent of damage caused by free radicals might be mitigated via supplementation with one or more antioxidants (6).

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases (2); however, recent concern has been paramount regarding the potential detrimental side effects of synthetic additives in humans (2). The 2 most commonly used synthetic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been restricted because they are toxic and cause DNA damage (7,8). Moreover, allopathic drugs frequently have negative side effects (9), whereas herbal medicines are comparatively much safer in this respect. Thus, natural antioxidants from plant sources have attracted much interest due to their safety (2).

Extensive ethnobotanical research has focused on the discovery of valuable drugs during the past few decades (10). Diverse medicinal plants have been screened and assessed for their ability to agonize free-radical-induced oxidative stress (1,11). Traditional medicinal plants are often economically favorable, locally available, and easily consumable substitutes for commercial medicines. Approximately 60%-80% of the world's population still relies on traditional medicines for the treatment of common illnesses (11). However, there has been little research about their effects on cellular macromolecules.

The present study investigated the potential to prevent DNA damage of 56 extracts prepared from 14 tribal medicinal plants, and estimated their antioxidant potential. All the selected plants are traditionally used as tribal medicines by several tribes, including the Irular in the Marudhamalai Hills (11°30′N, 77°30′E, Tamil Nadu, India) (12), the Malayali tribe in the Servarayan Hills (11°48′N, 78°11′E, Yercaud, Salem district, Tamil Nadu, India) (13), the Sonaghati tribe (23°45′-24°30′N, 82°45′-83°23′E, Sonbhadra district, Uttar Pradesh, India) (14), the Bhil, Meena, Garasia, Damor, and Kathodia tribes of Rajasthan (India) (15), and by several other ethnic groups in India.

This study aimed to evaluate the therapeutic potential, and screen the polar and non-polar extracts of 14 tribal medicinal plants, with respect to antioxidant properties and protection against oxidative stress-generated genotoxicity in vitro.

Materials and methods

Chemicals and reagents

Chemicals used in the study included 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) (purchased from Himedia Laboratories Pvt., Ltd. India), trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) and 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (procured from Sigma Aldrich Chemical Co., Milwaukee, WI, USA), and pBR322 (obtained from Medox Biotech India Pvt., Ltd., India). Other chemicals and solvents used were of standard analytical grade and HPLC grade, respectively.

Plant material

Whole plants were collected from Vellore (12°55′N, 79°11′E) and Marudhamalai Hills regions near Coimbatore (11°2′N, 77°2′E), Tamil Nadu, India, and were identified at the Botanical Survey of India, Southern Circle, Coimbatore, India. All the plants are freely available herbal crops; therefore, authorization was not required from the Indian government for their collection in compliance with the United Nations Convention on Biodiversity. Voucher specimens are maintained at our laboratory for future reference. Table 1 shows the botanical names and families, and the medicinal uses (among the tribes in the collection areas) of the plants.

Table 1. List of tribal medicinal plants investigated and their respective ethnic medicinal uses.

	Medicinal plant	Tribal medicinal use
1	*Abutilon indicum* (Linn.) Sweet Family: Malvaceae	Juice extracted from crushed leaves taken to ease parturition (12). Leaf juice mixed with jaggery and taken internally to treat snakebite (16). Root infusion given to cure fever, dry cough, and bronchitis. Seed powder used orally with water as aphrodisiac and laxative (14).
2	*Acalypha indica* Linn. Family: Euphorbiaceae	Juice extracted from crushed whole plant used to cure bronchitis in children (12). Leaf paste used externally as antidote for snake venom (16). Leaf paste taken internally to treat skin diseases (17). Whole plant extract used to heal wounds (18).
3	*Achyranthes aspera* Linn. Family: Amaranthaceae	Leaf juice applied externally for scorpion bite (13). Whole plant extract is used as anti snake venom (16). Root used to ease parturition and also to cure jaundice (19).
4	*Amaranthus viridis* Linn. Family: Amaranthaceae	Leaves used as emmolient in scorpion sting (2). Root is considered diuretic, laxative, and galactagogue. The decoction is given for retention of urine and to treat gonorrhea. Root paste is applied for curing piles (20).
5	*Andrographis paniculata* (Burm. f.) Nees Family: Acanthaceae	Leaf decoction used to cure diabetes (12). Whole plant extract used to treat dyspepsia (21) and asthma (22). Whole plant is also used against snakebites in folk medicine (23).
6	*Boerhaavia diffusa* Linn. Family: Nyctaginaceae	Leaf paste applied on wounds to stop bleeding (12). Decoction of root taken orally in treatment of jaundice (24). Root juice used to treat asthma (22). Leaves are used for the treatment of skin diseases and as an antidote to snakebite (14).
7	*Cassia auriculata* Linn. Family: Caesalpiniaceae	Seed paste applied to cure skin diseases (12). Leaf paste applied on painful parts to cure joint pain and inflammation (24). Leaf decoction taken internally to arrest thirst during illness (17).
8	*Emilia sonchifolia* (Linn.) DC Family: Asteraceae	Leaf juice used to treat eye inflammation. Root used in treatment of diarrhea (12). Juice of leaves applied to treat toothache (25).
9	*Lawsonia inermis* Linn. Family: Lythraceae	Leaves used to dye/condition hair and to soften skin of hands. Young shoot used in leprosy (12). Leaves also used in treatment of skin diseases (stops bleeding) and teeth problems (26).
10	*Leucas aspera* (Willd.) Spreng Family: Lamiaceae	Leaf juice used to relieve from chronic headache. Root used to cure dental pain. (12). Fresh leaf juice added with honey taken orally to cure gonorrhea, sterility, spermatorrhea, and impotency (27). Whole plant paste taken orally as an antidote to snakebite. It also provides relief from fever and cures insect stings. Root paste is used to treat asthma when taken orally (14).
11	*Mimosa pudica* Linn. Family: Leguminosae	Whole plant paste applied externally as an antidote for snake venom (16). Leaf paste applied externally to treat psoriasis (17) and eczema (19). Fresh leaf juice used to cure impotency and spermatorrhea (27). Whole plant paste applied on cuts and wounds for healing (19).
12	*Phyllanthus amarus* Schum. & Thon. Family: Euphorbiaceae	Leaf juice administered to cure fever and jaundice. (12,17).
13	*Trianthema portulacastrum* Linn. Family: Aizoaceae	Root infusion given internally to relieve constipation (12). Leaf decoction used to treat asthma (22). Whole plant extract taken orally for contraction of the uterus and as antidote to alcohol reactions. Its roots are used as abortifacient, and for asthma, jaundice and obstruction of the liver (14).
14	*Vitex negundo* Linn. Family: Verbenaceae	Leaf infusion taken to cure asthma (12). Juice of fresh leaves applied externally to treat joint pain (24). Leaf juice also used as an antidote for snake venom (16).

Processing and extraction

Healthy plants were screened for contamination by other species and thoroughly washed. The cleansed plants were freeze dried for 2 months at –80 °C in an MDF-U32V V.I.P.™ Series –86 °C ultra-low temperature freezer (Sanyo Biomedical, IL, USA). The dried plants were powdered for the preparation of extracts. Whole-plant powder was serially extracted with hexane, chloroform, methanol, and water using a Soxhlet apparatus. The crude extracts were concentrated under reduced pressure (hexane extract: 360 mbar; chloroform extract: 474 mbar; methanolic extract: 337 mbar; aqueous extract: 72 mbar) with a Rotavapor R-215 (BÜCHI, Labortechnik AG, Switzerland) to yield dry extracts.

DPPH radical scavenging activity

The DPPH assay was performed according to the method of Brand-Williams et al. (28), with a few modifications. To 2 mL of extract solution made in methanol (at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL, respectively) 1 mL of $DPPH^{\bullet}$ solution (0.2 mM/mL methanol) was added and mixed vigorously. The mixture was incubated in the dark at 20 °C for 40 min. Absorbance was measured at 517 nm using a Cary 50 UV-Vis spectrophotometer (Varian, Inc., CA, USA) with methanol as a blank. Trolox was used as a positive control. IC_{50} values for the percentage of DPPH radical scavenging were estimated.

ABTS radical scavenging activity

The ABTS assay was performed according to the protocol of Arnao et al. (29). Different quantities (25, 50, 100, and 200 µg) of the phyto-extracts were tested. Absorbance was measured at 734 nm. IC_{50} values for the percentage of ABTS radical scavenging were calculated.

Ferric reducing antioxidant property (FRAP)

The FRAP assay was performed according to Benzie and Strain (30), with some modification. The stock solutions were 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 .3H_2O$ and 16 mL $C_2H_4O_2$; pH 3.6), TPTZ solution (10 mM TPTZ in 40 mM HCl), and 20 mM $FeCl_3.6H_2O$ solution. The working FRAP solution was prepared freshly by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3.6H_2O$ solution, and then warmed to 37 °C before use. Then, 150 µL of individual extract solutions (containing 25, 50, 100, and 200 µg of extracts, respectively) was allowed to react with 2.85 mL of FRAP solution for 30 min in the dark. Absorbance was measured at 593 nm. IC_{50} values for the percentage of Fe^{+3} ion reduction were evaluated.

DNA damage inhibition efficiency

The potential of each extract to prevent DNA damage was tested by photolyzing pBR322 plasmid DNA via UV radiation in the presence of H_2O_2 and performing agarose gel electrophoresis with the irradiated DNA (31). Into polyethylene microcentrifuge tubes, 1-µL aliquots of pBR322 (200 µg/mL) were placed, and then 50 µg of each of the 56 extracts was separately added to individual tubes. One tube did not have any extract added to it, which served as the irradiated control (C_R). Into all the tubes, 4 µL of 3% H_2O_2 was added (including C_R), and then they were placed directly on the surface of a UV transilluminator (300 nm). The samples were irradiated for 10 min at room temperature; 1 µL aliquot of stock pBR322 plasmid DNA was placed in a separate tube and served as the non-irradiated control (C_0). All DNA was run on 1% agarose gel and photographed with a Lourmat gel imaging system (Vilbar, France).

Statistical analysis

All analyses were carried out in triplicate. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA to determine the significance of differences between the groups at $P < 0.05$. MATLAB v.7.0 (Natick, MA, USA), SPSS v.9.05 (Chicago, IL, USA), and Microsoft Excel 2007 (Roselle, IL, USA) were used for statistical and graphical evaluations.

Results and discussion

Floral resources have been extensively used to design drugs as antitheses of free radicals (1). To extrapolate such prospective clinical use, plants have been thoroughly evaluated for their antioxidant potential, thereby confirming their roles as potent precursors for developing effective medicines that protect against oxidative stress (2).

DPPH radical scavenging activity

DPPH$^{\bullet}$ is a stable free radical that has been used extensively to determine the free radical scavenging ability of various compounds, as a measure of their antioxidant potential (1,2). It has a maximum absorbance at 517 nm. Absorbance decreases when antioxidants donate protons to DPPH$^{\bullet}$, thereby reducing the latter (28). In the present study quantitative examination showed that there was a general trend of significantly ($P < 0.05$) higher DPPH$^{\bullet}$ radical scavenging activity by the polar (aqueous and methanolic) extracts, as compared to the non-polar (hexane and chloroform) extracts. IC_{50} values are shown in Table 2 and are ranked according to radical scavenging activity; lower IC_{50} values represent higher radical scavenging potential.

Table 2. DPPH assay (IC_{50}). The ranks are with respect to IC_{50} values which are expressed as mean ± SD (n = 3, $P < 0.05$).

Rank	Extract	IC_{50} ± SD (in µg/mL)	Rank	Extract	IC_{50} ± SD (in µg/mL)
1.	*Lawsonia inermis* aqueous	32.27 ± 0.35	29.	*Leucas aspera* aqueous	427.99 ± 2.50
2.	*Lawsonia inermis* methanolic	32.87 ± 0.44	30.	*Emilia sonchifolia* aqueous	446.03 ± 0.22
3.	*Phyllanthus amarus* methanolic	52.14 ± 0.63	31.	*Abutilon indicum* chloroform	452.99 ± 0.41
4.	*Phyllanthus amarus* aqueous	63.50 ± 0.77	32.	*Phyllanthus amarus* hexane	454.34 ± 0.15
5.	*Mimosa pudica* methanolic	67.34 ± 0.49	33.	*Acalypha indica* methanolic	483.87 ± 1.21
6.	*Vitex negundo* methanolic	80.45 ± 2.15	34.	*Mimosa pudica* hexane	494.62 ± 1.21
7.	*Vitex negundo* aqueous	93.73 ± 1.84	35.	*Acalypha indica* aqueous	527.86 ± 0.63
8.	*Lawsonia inermis* chloroform	99.92 ± 0.56	36.	*Achyranthes aspera* hexane	559.32 ± 1.25
9.	*Leucas aspera* methanolic	119.97 ± 1.71	37.	*Trianthema portulacastrum* methanolic	578.72 ± 0.60
10.	*Cassia auriculata* chloroform	148.25 ± 0.73	38.	*Leucas aspera* hexane	617.95 ± 0.54
11.	*Cassia auriculata* aqueous	152.50 ± 0.61	39.	*Abutilon indicum* aqueous	644.97 ± 0.93
12.	*Achyranthes aspera* aqueous	179.35 ± 0.87	40.	*Achyranthes aspera* chloroform	652.60 ± 0.60
13.	*Vitex negundo* chloroform	190.58 ± 1.33	41.	*Andrographis paniculata* hexane	720.63 ± 0.23
14.	*Mimosa pudica* aqueous	191.67 ± 0.64	42.	*Trianthema portulacastrum* hexane	722.56 ± 0.27
15.	*Boerhaavia diffusa* aqueous	200.82 ± 0.55	43.	*Andrographis paniculata* chloroform	758.37 ± 0.52
16.	*Emilia sonchifolia* methanolic	203.89 ± 0.58	44.	*Acalypha indica* hexane	805.27 ± 0.25
17.	*Amaranthus viridis* aqueous	236.06 ± 0.20	45.	*Andrographis paniculata* aqueous	825.76 ± 0.11
18.	*Mimosa pudica* chloroform	253.50 ± 1.56	46.	*Amaranthus viridis* methanolic	828.67 ± 0.76
19.	*Abutilon indicum* methanolic	280.88 ± 0.94	47.	*Emilia sonchifolia* hexane	892.69 ± 1.83
20.	*Lawsonia inermis* hexane	286.91 ± 0.28	48.	*Vitex negundo* hexane	913.76 ± 2.47
21.	*Cassia auriculata* methanolic	288.39 ± 0.63	49.	*Trianthema portulacastrum* aqueous	939.08 ± 0.40
22.	*Achyranthes aspera* methanolic	291.92 ± 0.34	50.	*Abutilon indicum* hexane	1038.20 ± 0.28
23.	*Emilia sonchifolia* chloroform	294.73 ± 0.47	51.	*Trianthema portulacastrum* chloroform	1125.90 ± 0.23
24.	*Boerhaavia diffusa* methanolic	327.40 ± 0.68	52.	*Cassia auriculata* hexane	1158.40 ± 0.91
25.	*Amaranthus viridis* chloroform	341.67 ± 0.67	53.	*Amaranthus viridis* hexane	1305.60 ± 1.20
26.	*Phyllanthus amarus* chloroform	367.28 ± 0.81	54.	*Leucas aspera* chloroform	1322.60 ± 0.28
27.	*Andrographis paniculata* methanolic	370.75 ± 0.85	55.	*Acalypha indica* chloroform	1688.00 ± 0.62
28.	*Boerhaavia diffusa* chloroform	409.81 ± 0.62	56.	*Boerhaavia diffusa* hexane	2351.60 ± 0.18

ABTS radical scavenging activity

The ABTS assay was performed with the 10 extracts that had the highest activity according to the DPPH assay. Table 3 shows the IC_{50} values for scavenging the $ABTS^{\bullet+}$ radical. Although a strong similarity to the DPPH assay results was observed, the ranking of the top 10 extracts for ABTS was somewhat different than that of the DPPH IC_{50} values. The results of these radical scavenging assays (DPPH and ABTS) suggest that the high-ranking extracts might prevent reactive radical species from damaging such biomolecules as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, amino acids, proteins, and sugars in susceptible biological and food systems (32).

Ferric reducing antioxidant property (FRAP)

Similar to ABTS assay, the FRAP assay was performed with the 10 extracts that had the highest activity according to the DPPH assay. The IC_{50} values and rankings for these extracts are presented in Table 3. The differential scavenging activity of the extracts against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and Fe^{+3} radicals might have been due to the different mechanisms of the radical-antioxidant reactions in the 3 assays. The stoichiometry of reactions between the antioxidant compounds in the extracts and the $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and Fe^{+3} radicals are quite dissimilar, which may account for the observed differences in their scavenging potential. The diversity of radical scavenging shown with these assays might also be due to such factors as stereoselectivity of the radicals or the differential solubility of the extracts in the 2 testing systems (33); therefore, the lack of any significant correlation between the 3 models may be justified in the case of these crude extracts, each of which contains a variety of antioxidants.

Accordingly, cumulative analysis of the results of all 3 antioxidant assays ($DPPH^{\bullet}$, $ABTS^{\bullet+}$, and Fe^{+3}) was performed by calculating the cumulative rank score (not shown) for each of the top 10 extracts with maximum radical-scavenging potential. Table 3 shows the cumulative ranking of the top 10 extracts.

The constituents of the hexane, chloroform, methanolic, and aqueous crude extracts of the selected medicinal plants differentially scavenged different free radicals ($DPPH^{\bullet}$, $ABTS^{\bullet+}$, and Fe^{+3}), and hence might exert protective effects against oxidative damage to such biological macromolecules as lipids, proteins, and DNA in varying degrees.

DNA damage inhibition efficiency

Diverse plant resources were observed to protect DNA from oxidative stress due to UV-induced photolysis of H_2O_2 (1,2,31). UV-photolysis of H_2O_2 generates $^{\bullet}OH$ radicals, which cause colossal oxidative damage. $^{\bullet}OH$ bound to DNA leads to strand

Table 3. Cumulative ranks of the 10 extracts having maximum antioxidant efficiency (cumulative) in DPPH, ABTS, and FRAP assays. The ranks are with respect to IC_{50} values which are expressed as mean ± SD (n = 3, P < 0.05). (Note: DPPH ranks are according to Table 2).

Cumulative rank	Extract	DPPH Rank	ABTS assay		FRAP	
			Rank	IC_{50} (in µg)	Rank	IC_{50} (in µg)
1	*Lawsonia inermis* aqueous	1	1	12.57 ± 0.35	1	313.93 ± 0.39
2	*Phyllanthus amarus* aqueous	4	2	12.58 ± 0.44	2	392.37 ± 0.67
2	*Lawsonia inermis* methanolic	2	3	12.59 ± 0.63	3	430.8 ± 0.35
4	*Phyllanthus amarus* methanolic	3	5	16.56 ± 0.49	4	448.81 ± 0.98
5	*Vitex negundo* aqueous	7	4	13.01 ± 0.77	7	1021.38 ± 0.95
5	*Vitex negundo* methanolic	6	7	23.25 ± 1.84	5	476.91 ± 1.29
7	*Leucas aspera* methanolic	9	6	21.45 ± 2.15	6	935.67 ± 1.1
8	*Lawsonia inermis* chloroform	8	8	41.42 ± 0.56	8	1815.67 ± 0.11
8	*Mimosa pudica* methanolic	5	10	65.4 ± 0.73	9	2499.85 ± 2.1
10	*Cassia auriculata* chloroform	10	9	42.96 ± 1.71	10	5306.79 ± 1.39

breakage, deoxysugar fragmentation, and base modification. Moreover, oxidation of lipids induced by $^{\bullet}OH$ and other ROS can generate end products, such as malondialdehyde and unsaturated aldehydes, that can attach to DNA and produce mutagenic adducts (34).

Figure 1 shows the electrophoretic pattern of pBR322 DNA following UV-photolysis of H_2O_2 in the absence (in controls C_0 and C_R) and presence of the extracts in samples H (treated with hexane extract), C (treated with chloroform extract), M (treated with methanolic extract), and A (treated with aqueous extract) for each plant. Normal pBR322 (C_0) showed 2 bands with agarose gel electrophoresis. The faster moving band represented the native form of supercoiled circular DNA (scDNA) and the slower moving band corresponded to the open circular form (ocDNA) (19). C_R did not show any bands, which indicates that the entire DNA was degraded.

UV-photolysis of H_2O_2 in the H, C, M, and A samples exhibited differential banding patterns, inferring that different extracts of the tested plants

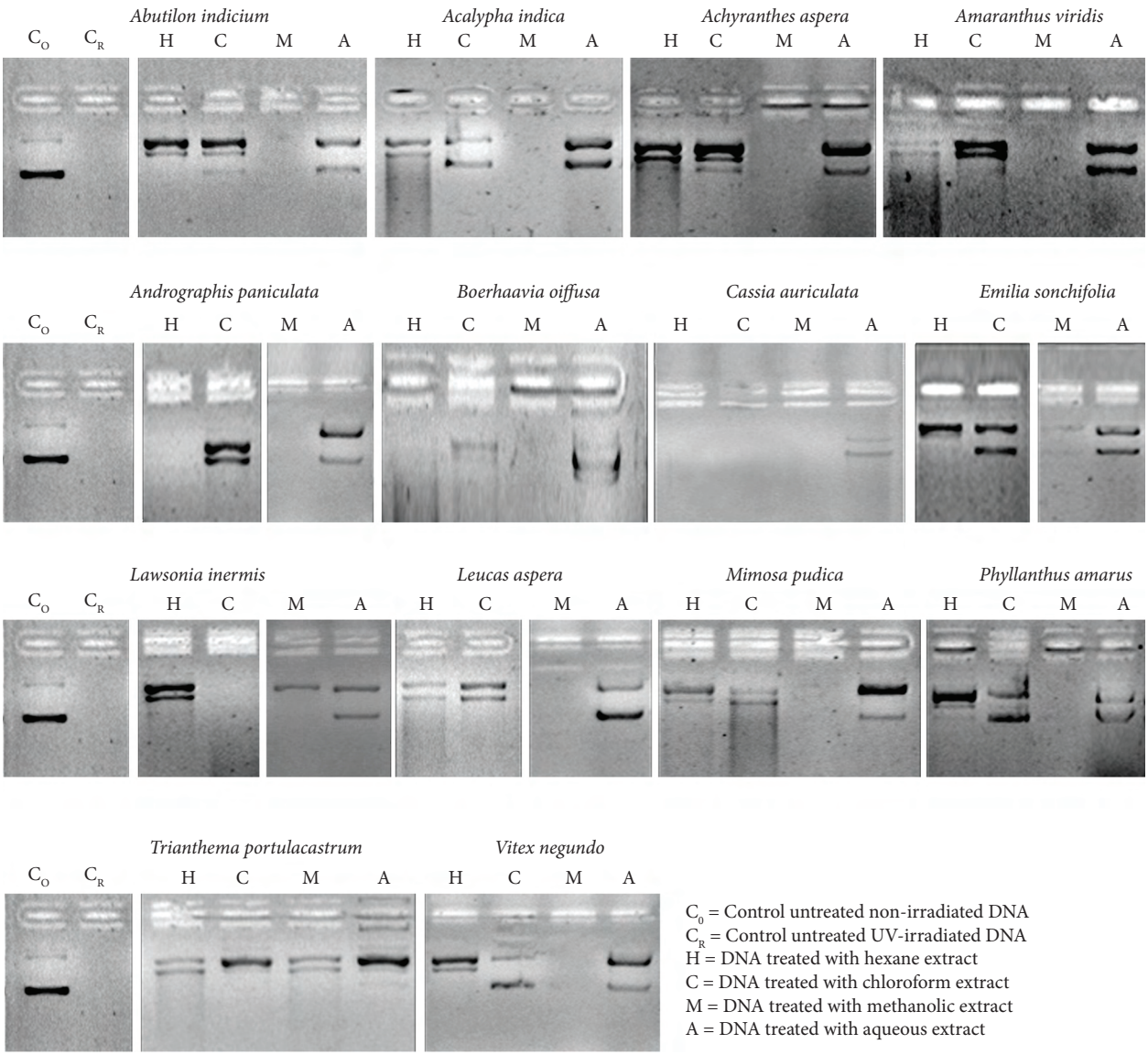

Figure 1. Effects of the tested extracts (50 µg) against oxidative damage to DNA (pBR322) caused by UV-photolysis of H_2O_2 (3%, v/v).

exhibited diverse magnitudes of DNA protection against oxidative stress. Plant extracts with a DNA banding pattern identical to C had maximum protective potential. With intermediate patterns, in which the scDNA or ocDNA was not visible or only slightly visible, or a new intermediate linear DNA (linDNA) band appeared, the extract exhibited partial/negligible DNA protection. Samples that did not show any bands contained extracts that did not have the ability to inhibit oxidative DNA damage.

Figure 2 shows the percentage of difference in DNA protection of all 56 extracts. In all, 27% of the extracts exhibited extensive protection, 37% exhibited partial DNA protection, and 7% exhibited negligible protection, while the remaining 29% provided no protection to DNA.

Considering only the aqueous extracts, 78.56% exhibited extensive DNA protection, 7.16% exhibited partial protection, and 14.28% exhibited negligible protection. Hexane extracts were similar to the aqueous extracts, in terms of protective activity. Although none of the hexane extracts exhibited extensive DNA protection, 78.56% exhibited the potential for partial protection and the remaining 21.44% did not have any protective ability. Among the chloroform extracts, 28.56% extensively protected DNA against oxidative damage, 50% exhibited partial protection, 7.16% exhibited negligible protection, and the remaining 14.28% exhibited no protection. The methanolic extracts exhibited the least protection to pBR322 against oxidative damage. None of the methanolic extracts exhibited total protection to DNA; only 14.28% exhibited partial DNA protection, 7.16% exhibited very negligible protection, and 78.56% exhibited no protective activity. Furthermore, the top 3 extracts (*Lawsonia inermis* aqueous, *Phyllanthus amarus* aqueous, and *Lawsonia inermis* methanolic), according to the cumulative ranking of the DPPH, ABTS, and FRAP assays (Table 3), provided maximal DNA protection against oxidative damage. The evaluation results for antioxidant potential and DNA protection correlated well, and as such protection against oxidative DNA damage might have been due to the high antioxidant potential of these 3 extracts.

Experimental evidence showing that 27% of the extracts exhibited considerable plasmid DNA protection against a high level of H_2O_2-driven oxidative damage suggests that treatment with these extracts might reduce oxidative damage to mitochondrial and genomic DNA (1), mitigating the development and/or progression of degenerative ailments related to oxidative cell/tissue damage. In conclusion, these tribal medicinal plant extracts might

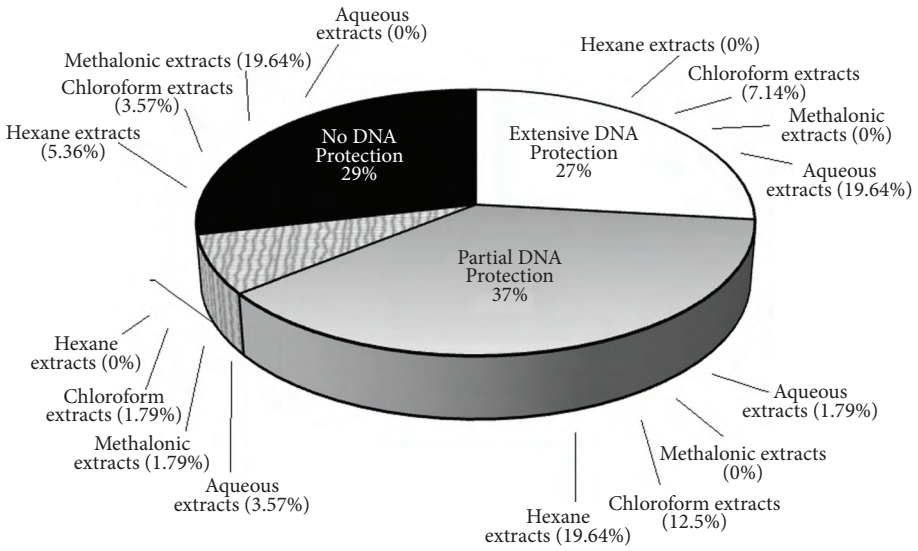

Figure 2. Efficiency of different polar and non-polar extracts with respect to varying degrees of DNA protection against oxidative damage.

be used as prophylactics against a host of degenerative diseases caused by free radicals. Additional research on all these medicinal plants and simultaneous structural elucidation of the active isolates are warranted.

Acknowledgement

The authors thank the management of VIT University, Vellore, Tamil Nadu, India, for providing the facilities necessary to conduct this study.

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