ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Study of Antioxidant, Anti-inflammatory, and DNA-Damage Protection Properties of Some Indian Medicinal Plants Reveal their Possible Role in Combating Psoriasis

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Abstract

Reactive oxygen species (ROS) are generated due to severe oxidative stress, thereby resulting in pathogenesis of various disorders in humans, including psoriasis. DNA damage is the major manifestation of long term ROS exposure. ROS can be scavenged by natural antioxidant compounds present in medicinal plants. In this study, aqueous, methanolic and chloroform extracts of eleven dermatologically significant Indian medicinal plants were evaluated for their ROS scavenging and antioxidant properties, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power assay (FRAP), along with total phenolic content determination. Lipoxygenase inhibition assay was conducted to determine anti-inflammatory activities. DNA-damage protective capacities were also assessed using plasmid pBluescript II SK(–) DNA, where DNA damage was induced by Fenton reaction. The study reveals that the extracts of *T. cordifolia*, *C. paniculatus*, *C. longa* and *A. vera* performed far much better, in almost all the assays, with regards to *P. corylifolia*, a medicinal plant traditionally used to treat psoriasis, thus indicating these plants to be potent sources of effective drug formulation for treatment of psoriasis.

Keywords: Reactive oxygen species; Psoriasis; Antioxidants; Anti-inflammatory; Oxidative DNA damage.

Abbreviations: ROS: Reactive oxygen species; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant potential; TEAC: Trolox equivalent antioxidant capacity; TPC: Total phenolic content; DNA: Deoxyribonucleic Acid; LOX: Lipoxygenase; PUFA: Polyunsaturated fatty acids; GAE: Gallic acid equivalent; NDGA: Nordihydroguaiaretic acid

Introduction

Skin, the outermost covering of the whole body, is prone to a lot of oxidative stresses leading to elevated production of unstable molecules known as reactive oxygen species (ROS), such as O_2^-, H_2O_2 and •OH, which are highly toxic to the cells of human body. Free radicals are highly reactive molecules that are formed as a result of intrinsic and extrinsic factors due to by-products of normal metabolism and environmental stressors respectively. Free radicals damage lipid-rich membranes, cellular DNA and proteins present in the cells. They also alter cell cycles and influence the release of cytokines (pro-inflammatory mediators), that are implicated in the pathogenesis of various inflammatory skin diseases. Reports suggest that these free radicals may participate in the pathogenesis of allergic reactions in the skin (Bandyopadhyay *et al*., 1999).

Psoriasis is a chronic dermatological autoimmune disorder occurring in people of all ages and is characterized by hyper-proliferation and inflammation of the skin. Oxidative stress is considered as one of the important etiopathological factors responsible for the development and exacerbation of psoriasis. According to the reports, in case of psoriasis it has been observed that there exists an imbalance in the oxidant-

antioxidant system in the body. Studies also provide evidences of an increased ROS production and insufficient antioxidant activity in psoriatic lesions (Zhou et al., 2009; Samuel and Murari, 2013). Moreover, ROS are known to have deleterious effects on the DNA present in the cells of the body. They induce numerous lesions in DNA by causing deletions, mutations and other lethal genetic effects, thus severely damaging it (Mittova, 2000).

Another important factor that contributes towards the exacerbation of psoriasis is the inflammatory action of Lipoxygenases (LOXs) present in the body. LOXs are enzymes that catalyze the addition of molecular oxygen to polyunsaturated fatty acid (PUFA) like Arachidonic acid (AA) to form specific unsaturated fatty acid hydroperoxide derivatives (Brash, 1999). These LOX products have been reported to have an implication in psoriasis and have been found to be present in psoriatic lesions (Hammarstrom, 1975; Camp, 1983).

ROS are generally curbed by innumerable antioxidants present in the human body (Halliwell and Gutreridge, 1989). However, an over-exposure to deleterious oxidative stressors presents a challenge to the cellular antioxidant systems. Hence, an external supply of antioxidants becomes inevitable for up-regulation of defense systems by increasing ROS scavenging activity, reducing inflammation and stimulating immunity. Moreover, antioxidant strategies have proven to be beneficial therapeutics in psoriasis (Zhou et al., 2009).

Medicinal plants are a rich source of antioxidants that can target oxidative stress thereby protecting skin from damaging ROS activity and providing a safer and cost effective way to treat skin diseases. In this study, eleven medicinal plants with beneficial dermatological properties were screened for their antioxidative, free radical scavenging, and anti-inflammatory properties, along with total phenolic content determination of their aqueous, methanolic, and chloroform extracts. DNA damage preventive capacities of the crude extracts of the selected plants were also evaluated, against pBluescriptII SK(–) DNA strand scission by Fenton reaction generated •OH radicals. The bioactivities of the selected plants were assessed against *P. corylifolia*, a plant used since ages to cure dreadful skin diseases like leukoderma, leprosy, and most importantly, psoriasis (Sah *et al*., 2006), in order to evaluate their potential psoriasis preventive capacities. This study provides an insight into the possible role of some of the selected medicinal plants in the treatment of psoriasis.

Materials and Methods

Plant Material

Different plant parts of the eleven medicinally important plants were selected for the study. These were *Aloe vera* (Leaves), *Amaranthus hypochondriacus* (Seeds), *Andrographis paniculata* (Leaves), *Artemisia annua* (Leaves), *Azadirachta indica* (Leaves), *Celastrus paniculatus* (Seeds), *Curcuma longa* (Root), *Nigella sativa* (Seeds), *Psoralea corylifolia* (Seeds), *Tinospora cordifolia* (Stem), and *Triticum aestivum* (Leaves). All the plants were identified and authenticated by the taxonomists of the Ayurvedic department of Banaras Hindu University and voucher specimens were deposited there.

Preparation of Plant Extracts

Plant material from each plant was weighed to 10 g and coarsely ground using pestle and mortar. The ground powder of each plant material was extracted in 100 ml of the desired solvent to prepare the respective solvent extract. The solvents used were distilled water, methanol, and chloroform. The prepared extract was subjected to centrifugation at 4000g for 30 min at 25°C. The supernatant was evaporated to dryness at 40^{0}C for 2-4 days to form a thick concentrated extract. The extracts were preserved at 4°C in brown bottles until use.

Antioxidant Activity Determination

DPPH radical scavenging assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay was used to evaluate the free-radical scavenging activity of the various solvent extracts of the selected plants. 10 µl of each solvent extract was added to 100 µl of 0.2 mM methanolic solution of DPPH (Sigma-Aldrich). After vigorous shaking of the reaction mixture, it was incubated at 25°C for 5 min. The absorbance of the mixture was measured at 520 nm. Percent inhibition of DPPH free radical was calculated as:

$$\% \text{ Inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100$$

($A_{control}$ = Absorbance of Control; A_{sample} = Absorbance of Sample)

Ferric reducing antioxidant potential

FRAP assay was determined using freshly prepared FRAP reagent, which was warmed in a water bath at 37°C before use. 100 µl of each plant extract was treated with 3 ml freshly prepared FRAP reagent. The reaction mixture was incubated for 4 min at 25^{0}C, thereafter absorbance was measured at 593 nm. FRAP values of the samples were calculated using the standard curve of $FeSO_4$ solution. Results were expressed as µmol Fe(II)/g dry weight of plant extract.

Trolox equivalent antioxidant capacity

TEAC assay was used to determine the free radical scavenging capacity of the prepared solvent extracts using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation. 100 µl of the sample was mixed with 4 ml ABTS working solution (7 mM ABTS stock solution in 2.45 mM potassium persulfate, 1:1 ratio). The reaction mixture was incubated for 5 min at room temperature and the absorbance was measured at 734 nm. Trolox solution (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid,

Sigma-Aldrich) was used as an antioxidant standard. The results were expressed as μmol Trolox/g dry weight of plant extract.

Total phenolic content determination

Folin–Ciocalteu method was used to evaluate total phenolic content (TPC) of the plant extracts. 200 μl of each test sample was mixed with 500 μl of Folin-Ciocalteu reagent (Sigma-Aldrich). After incubating the mixture for 3 min, 2 ml of 20% Na_2CO_3 solution was added. The reaction mixture was then vortexed, followed by boiling for 1 min in a water bath and then cooling. The absorbance was measured at 650 nm. Gallic acid (Sigma-Aldrich) served as the reference standard, and the results were expressed as mg GAE/g (mg Gallic acid equivalent/g) dry weight of plant extract.

Anti-Inflammatory Capacity Determination

Lipoxygenase Inhibition Assay

Anti-inflammatory activity of the plant extracts were estimated by using LOX inhibition assay. The test plant extracts were incubated with LOX enzyme solution for 5 min at 25° C, thereafter initiating the reaction by adding linoleic acid (substrate) and 0.2 M borate buffer (pH 9.0) to the mixture. After incubating the mixture at 25 °C for 5 min, the absorbance of the reaction mixture was measured at 234 nm. NDGA (Nordihydroguaiaretic acid, Cayman Chemical) served as a positive control. Lipoxygenase percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100$$

($A_{control}$ = Absorbance of Control; A_{sample} = Absorbance of Sample)

DNA Damage Inhibition Efficiency

DNA protection capacities of the test plant extracts were performed by using pBluescript II SK(–) DNA. The plasmid DNA was isolated using a QIAprep Spin Mini prep Kit according to the manufacturer's instructions. pBluescript II SK(–) (250 ng) was incubated with 0.5 mM $FeSO_4$, 25 mM H_2O_2 and 50 mM phosphate buffer (pH 7.4) in the presence or absence of plant extracts (0.2 μg/ml). The total reaction mixture of 12 μl was incubated for 1 h at 37°C. The test samples were then analyzed on 1% agarose gels at 70 V at room temperature to analyze the extent of DNA damage by Fenton's reaction and DNA protective capacities of the plant extracts. 1 mM Quercetin (Cayman Chemical) served as a positive control. Gels were scanned using Gel documentation system and the densitometric analysis of the bands was performed using Quantity One analysis software (Bio-Rad). The native form of DNA that moved faster represented supercoiled DNA (scDNA), whereas the damaged form of DNA that moved slower represented open circular DNA (ocDNA).

Statistical Analysis

All the experiments were performed in triplicate and the data were expressed as Mean ± Standard error (SE). Microsoft Excel (2007), SPSS (16.0) and Sigma Plot (v.11.0) were used for statistical and graphical evaluations.

Results and Discussion

Medicinal plants that are rich in a wide variety of chemical compounds like antioxidants and polyphenols, when consumed by humans, exhibit remarkable therapeutic properties. Eleven medicinal plants that were studied are enlisted in Table 1, with their respective parts used and their various roles in curing skin diseases.

Antioxidant Potential

The free radical scavenging activity of the various plant extracts was analyzed using DPPH radical. DPPH is a stable free radical that gets reduced into a yellow colored compound Di-phenyl hydrazine upon reaction with the antioxidants present in the plants. It thus gives the measure of the antioxidant potential of the test samples. In this assay, as shown in Fig. 1(a), it was observed that the polar extracts (aqueous and methanolic) of all the plants, in general, showed strong scavenging potential with respect to the non-polar (chloroform) extract. This finding is in accordance with the study performed by Oboh *et al.* (2008). Out of the eleven plants, five plants namely, *A. vera*, *A. hypochondriacus*, *A. paniculata*, *C. paniculatus*, and *N. sativa,* showed higher scavenging potential in their aqueous extracts, while the other five, i.e., *A. indica*, *C. longa*, *P. corylifolia*, *T. cordifolia*, and *T. aestivum* had maximum DPPH percent inhibition in their methanolic extract. However, chloroform extract of only one plant, i.e. *A. annua* was found to be having higher free radical scavenging potential. Moreover, the assay suggests *C. paniculatus* and *A. paniculata* to be the best free radical scavengers with all their three solvent extracts performing equally well. Incidentally, the *in-vivo* studies conducted on mice with these plants also demonstrated them to be strong free radical scavengers (Godkar *et al*., 2006; Tripathi and Kamat, 2007).

FRAP assay was another test employed to check the antioxidant potential of the plant extracts. The principle of this assay is based on the fact that the antioxidants present in the plants reduce the ferric ions of the FRAP reagent to the ferrous form, which could be measured from the change in the absorbance. In Fig. 1(b), depicting FRAP values, a similar trend to that of DPPH assay is observed, wherein the polar solvent extracts performed better as antioxidants in comparison to non polar extracts. *A. vera*, *A. hypochondriacus*, *A. paniculata*, *A. indica*, and *N. sativa* had their aqueous extracts with best antioxidant capacities whereas the methanolic extract of *C. longa*, *P. corylifolia*, *T. cordifolia*, and *T. aestivum* had the maximum reduction potential. *A. annua* and *C. paniculatus*, however, were found to be the best antioxidants in their chloroform

extracts. Overall results of the assay suggest that *A. paniculata*, *A. hypochondriacus*, *A. annua*, *C. paniculatus*, and *C. longa* possess higher reducing potential among other plants. Furthermore, some other reports on these plants also demonstrate similar findings (Skowyra *et al*., 2014; Akinola *et al*., 2014; Arora and Rai, 2014).

ABTS radical cation was used in TEAC assay to evaluate free radical scavenging capacity of the plant extracts. The more the scavenging activity of an extract, the better is its antioxidant potential, which is calculated against the standard solution of Trolox. In Fig. 1(c), it is well evident that the aqueous extracts of seven out of eleven plants dominated as antioxidants, with respect to other extracts. These plants were *A. vera*, *A. paniculata*, *A. annua*, *A. indica*, *C. longa*, *T. cordifolia*, and *T. aestivum*. However, *A. hypochondriacus*, *N. sativa*, and *P. corylifolia* in their methanolic phases and *C. paniculatus* in its chloroform phase showed to have more antioxidant potential. Aqueous extract of *C. longa* exhibited the strongest scavenging potential, followed by aqueous extracts of *A. paniculata*, *A. annua*, *A. indica*, and *T. cordifolia*, thereby indicating polar extracts to be better antioxidants. Total phenolic content in the plants is determined to evaluate their free radical scavenging and antioxidant properties (Rice-Evans *et al.,* 1997). Fig. 2 depicts total phenolic content in various extracts of the selected plants. Maximum phenolic content was identified in *A. hypochondriacus* in its methanolic phase, which was followed by *A. indica*, *A. annua*, *P. corylifolia*, and *A. paniculata.* These results concur with other researchers who have also reported high phenolic content in these plants (Rosa *et al.*, 2009; Singh *et al*., 2005; Han *et al.,* 2008).

Table 1. Plants, their parts used in the study and their role in skin

Scientific Name	Common Name	Family	Part used	Role in skin	References
Aloe vera (L.) Burm. f.	Aloe	Aloaceae	Leaves	Used against inflammation; promotes wound healing; cures acne, rosacea, burns, bug bites	Davis *et al*., 1994; Haller, 1990
Amaranthus hypochondriacus L.	Chaulai	Amaranthaceae	Seeds	Improves skin quality; retards aging of skin, prevents development of wrinkles and damage due to harsh environment; oil used in dermatic diseases including herpes, non-healing ulcers, burns, dry eczema, dermatitis, mycosis, burns; rich source of vitamin E and vitamin C thus good for skin growth; possess antioxidant and anti inflammatory properties	López *et al*. 2011; Iqbal *et al*., 2012
Andrographis paniculata (Burm.f.) Wall. ex Nees.	Kalmegh	Acanthaceae	Leaves	Effective against acne, boils, abscesses, sores and eczema; used for irritated, itchy, inflamed and infected skin problems including fungal infections, wounds, ulcers and spots	Pole, 2006; Sheeza *et al*., 2006
Artemisia annua L.	Jwarharti	Asteraceae	Leaves	Useful in skin problems like athlete's foot, haemorrhoids, eczema, skin ulcers; effective against many fungal skin conditions; possess antibiotic, antioxidant, antiviral and antiulcerogenic properties	Juteau *et al*., 2002; Dia *et al*., 2001
Azadirachta indica A. Juss.	Neem	Meliaceae	Leaves	Used against skin diseases like dermatitis, eczema, acne, wounds, skin ulcers and infected burns; used as mosquito repellent; and skin softener; has antiviral, anti-fungal and anti-bacterial properties and used against microbial infections like ringworm	Chaturvedi *et al*., 2011; Mishra *et al*., 2013
Celastrus paniculatus Willd.	Malkangani	Celastraceae	Seeds	Used for the treatment of rheumatism, leprosy, eczema and other skin diseases; seed oil is used as a massage oil for the medicinal treatment of arthritis and inflammatory skin conditions	Handa, 1988; Arora and Rai, 2012

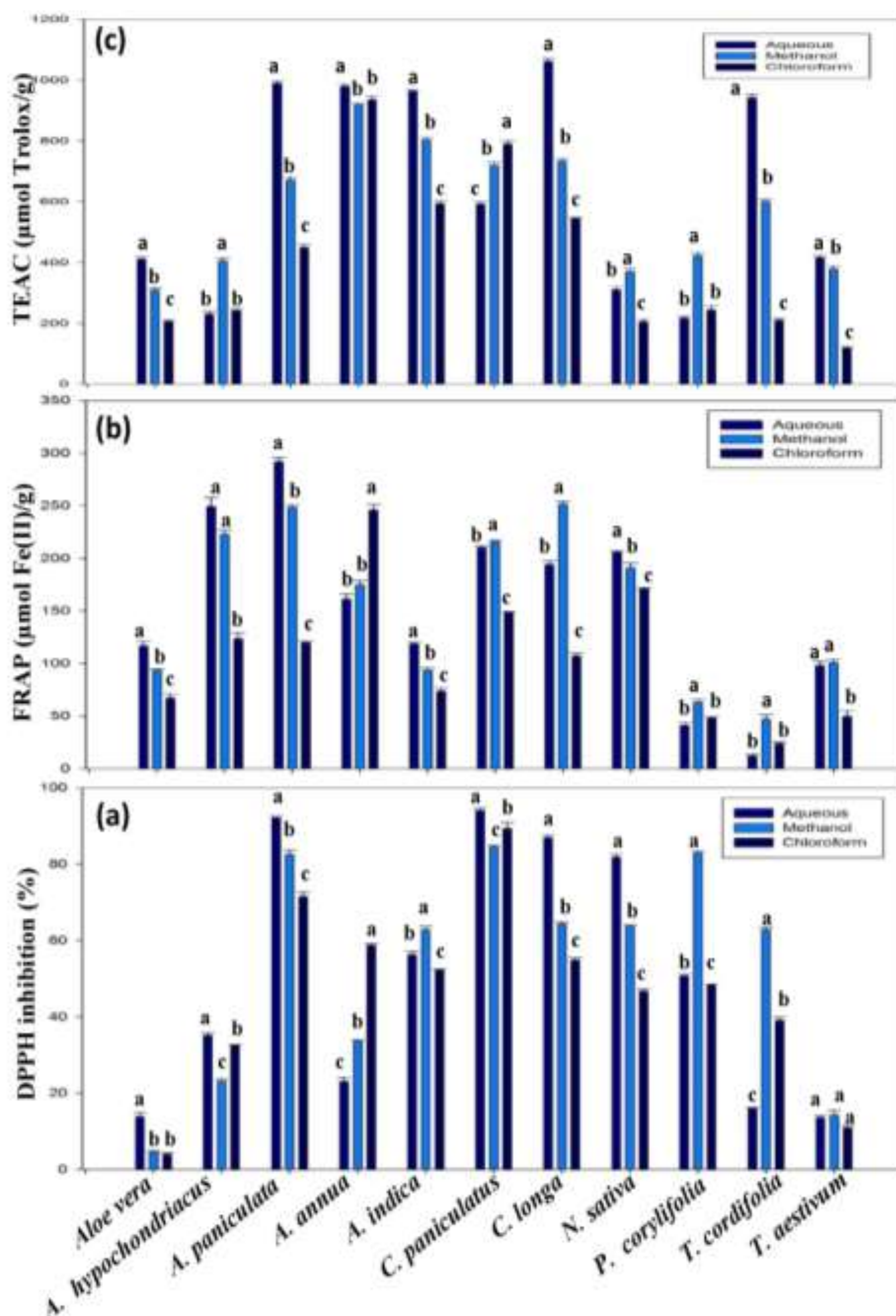

Fig. 1. Antioxidant potential of various extracts of the selected 11 plants (a) Free radical scavenging capacity, depicted as percent DPPH inhibition (b) Ferric reducing antioxidant power (FRAP) (c) Trolox equivalent antioxidant capacity (TEAC). Values are expressed as Mean ± SE (n=3).

Anti-Inflammatory Capacity

Lipoxygenase inhibition activity that evaluates the anti-inflammatory potential indicates that out of eleven plants, six were found to have higher inhibition efficiency in their chloroform extract, whereas the aqueous extract performed better in rest of the plants (Fig. 3). In the assay, *T. cordifolia* exhibited highest inhibition of 64.12±0.32 % (in its chloroform extract), followed by *C. longa*, *C. paniculatus*, and *A. vera.* These results are in accordance with other investigators who have also reported the lipoxygenase inhibitory activity in these plants (Kumar *et al*., 2011; Bezáková *et al*., 2005; Bezáková *et al*., 2014). Interestingly, all the three extracts of the aforementioned plants inhibited LOX equally well. Although aqueous extract of *P. corylifolia* showed a fourfold increment with respect to its other two solvent extracts, its inhibition capacity was lesser as compared to *T. cordifolia*, *A. vera*, *C. longa*, and *C. paniculatus*. The active solvent phases of all the plants in various assays are shown in Table 2.

Table 2: Plants with their active solvent extracts in the various assays

Plants	% DPPH inhibition	FRAP assay	TEAC assay	Phenolic contents	% LOX inhibition
Aloe vera	Aqueous	Aqueous	Aqueous	Aqueous	Chloroform
Amaranthus hypochondriacus	Aqueous	Aqueous	Methanolic	Methanolic	Aqueous
Andrographis paniculata	Aqueous	Aqueous	Aqueous	Methanolic	Aqueous
Artemisia annua	Chloroform	Chloroform	Aqueous	Methanolic	Chloroform
Azadirachta indica	Methanolic	Aqueous	Aqueous	Chloroform	Chloroform
Celastrus paniculatus	Aqueous	Chloroform	Chloroform	Methanolic	Aqueous
Curcuma longa	Methanolic	Methanolic	Aqueous	Chloroform	Chloroform
Nigella sativa	Aqueous	Aqueous	Methanolic	Chloroform	Aqueous
Psoralea corylifolia	Methanolic	Methanolic	Methanolic	Methanolic	Aqueous
Tinospora cordifolia	Methanolic	Methanolic	Aqueous	Methanolic	Chloroform
Triticum aestivum	Methanolic	Methanolic	Aqueous	Methanolic	Chloroform

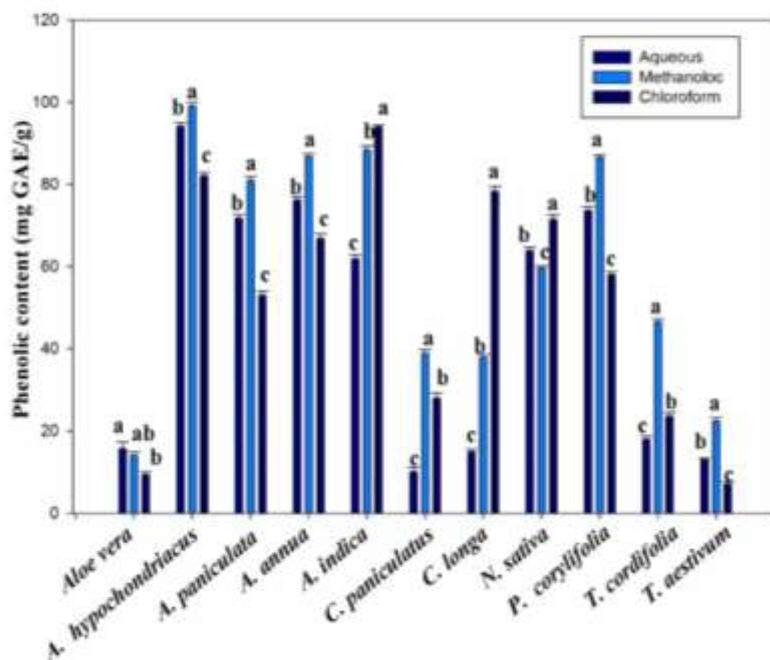

Fig. 2: Total phenolic content in various extract of the selected 11 plants.

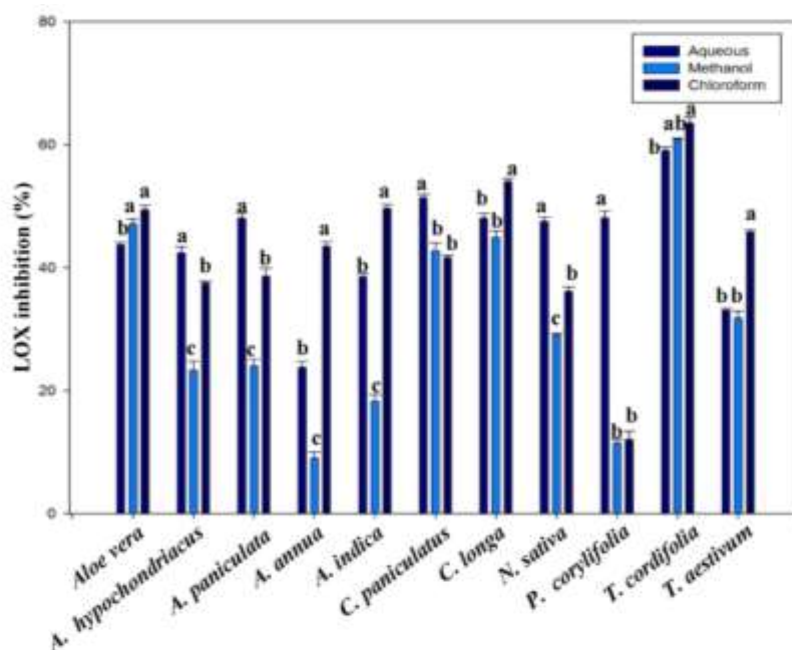

Fig. 3: Lipoxygenase inhibition (%) by three solvent extracts, aqueous, methanolic, and, chloroform, of the eleven selected plants. Values are expressed as Mean ± SE (n=3).

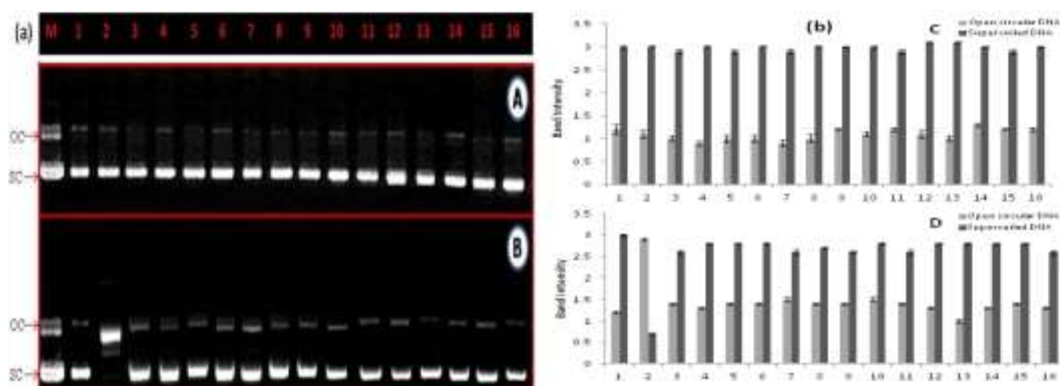

Fig. 4: (a). A. Electrophoresis patterns of pBluescript II SK (–) DNA showing equal amount of DNA in each lane, B. DNA breaks generated by •OH in the Fenton reaction and DNA damage protective effect of different plant extracts;
(b) Densitometric analysis of open circular and supercoiled DNA damage, corresponding to A & B are shown in C & D respectively. Lane 1: untreated control DNA (250ng); lane 2: $FeSO_4$ (0.5mM) + H_2O_2 (25mM) + DNA (250ng); lane 3: only H_2O_2 + DNA; lane 4: only $FeSO_4$ + DNA; lane 5: $FeSO_4$ + H_2O_2 + DNA + Quercetin (1mM); lanes 6-16: $FeSO_4$ + H_2O_2 + DNA + 0.2µg/ml aqueous extract each of *Artemisia annua*, *Curcuma longa*, *Celastrus paniculatus*, *Aloe vera*, *Amaranthus hypochondriacus*, *Andrographis paniculata*, *Psoralea corylifolia*, *Azadirachta indica*, *Nigella sativa*, *Triticum aestivum*, and *Tinospora cordifolia*.

DNA-Damage Protective Capacity

Fenton reaction was used *in vitro* to generate DNA strand scission in pBluescriptII SK(–) DNA. Aqueous extracts of all the eleven plants were used to evaluate their DNA-damage protective capacity against oxidative DNA damage. The electrophoresis patterns of pBluescript II SK (–) DNA shown in Fig. 3 depicts relative band intensity of ocDNA and scDNA. Higher the intensity of ocDNA, more is the damage inflicted on DNA. The untreated control DNA in lane 1 shows lesser ocDNA, whereas the scDNA is present in enormous amount. However, in lane 2, where DNA damage was caused by Fenton reaction, the band pattern of ocDNA and scDNA has inversed, with higher amount of the former and minimal amount of the latter. Fig. 4(a) shows the pattern of equally loaded DNA in all lanes, while Fig. 4(b) depicts the band patterns after Fenton reagent treatment. Although the densitometric analysis and electrophoresis patterns of all the plant extracts suggest having DNA damage protecting capacities, however *A. indica* possess maximum DNA damage prevention capacity, followed by *T. cordifolia* and *N. sativa,* which were highly efficient in protecting DNA as compared to *P. corylifolia,* with a superior or compatible effect to that of quercetin. DNA protective properties of these plants have also been previously reported by other researches (Manikandan *et al*., 2009; Ilaiyaraja and Khanum, 2011).

Conclusion

On analyzing the bioactivities of all the selected eleven plants in the current study, it is well evident that while polar solvent extracts of the plants generally performed well in antioxidant assays, the non-polar extracts exhibited better LOX inhibition potential and hence demonstrate good anti-inflammatory properties. The overall results suggest that amongst other potential plants used in the study, *T. cordifolia* was the most efficacious plant which exhibited good antioxidant potential, remarkable LOX inhibition efficiency and had equally good DNA protective capacity. Moreover, in all the evaluated bioactivities, the solvent extracts of *T. cordifolia* were far too efficient than *P. corylifolia*, a plant widely used in traditional medicine to treat psoriasis. Besides *T. cordifolia*, other plants whose extracts performed better than that of *P. corylifolia* in almost all the assays are *C. longa*, *C. paniculatus*, *A. vera*, *A. hypochondriacus*, and *A. indica.* Thus it can be concluded that these potent plants can effectively prevent the major causes of psoriasis in humans by reducing or alleviating oxidative stress, inhibiting LOX enzyme activity and protecting the cells of body from DNA damage. These plants can hence prove to have tremendous potential to be used in effective drug formulations to combat psoriasis.

Acknowledgement

The authors are grateful to DBT (Grant No. BT/PR13597/GBD/27/274/201) (Department of Biotechnology), Govt. of India, DST (PURSE) for financial support. Ms. Neha Arora, Neha Pandey and Deepika Tripathi express sincere gratitude to UGC (University Grants Commission), India, for financial assistance in the form of Research fellowship.

Conflict of Interest

The authors declare that there is no conflict of interest.

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