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Biochemical and antioxidant screening of three important medicinal plants from Dehradun, Uttarakhand

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Abstract
Physiological and antioxidant potential of important medicinal plants *i.e. Cassia fistula*, *Terminalia arjuna* and *Azadirachta indica* were determined. The radical scavenging activity of extracts was evaluated using 2, 2-diphenyl-1-picryhydrazyl (DPPH) assay and The total phenolic content was determined by following Folin-Ciocalteau assay. In the results different plants showed different activities, in the case of Chlorophyll a (*Chl a*) and chlorophyll b (*Chl b*) maximum recorded in *Azadirachta indica* and *Terminalia arjuna* respectively while minimum in *Cassia fistula*. In case of carotenoid and protein contents were found maximum in *Cassia fistula* and minimum in *Terminalia arjuna.* Antioxidant activity *i.e.*, DPPH and total phenolic contents were found maximum in *Terminalia arjuna* and minimum in *Cassia fistula.* These finding indicated that the *Terminalia arjuna* have most potent antioxidant potential as comparison with other two species. The present study reveals that the selected plants would exert beneficial effects by virtue of their antioxidant activity and could be harnessed as drug formulation.

Keywords: medicinal plants, antioxidant activity, DPPH, total phenols

1. Introduction
Medicinal plants have been used virtually in all cultures as a source of medicine. They include various types of plants used in herbalism. Active compounds produced during secondary metabolism are usually responsible for the medicinal properties of medicinal plants that used throughout the globe for various purposes, including treatment of infectious diseases [1]. Medicinal plants have unlimited ability to synthesize secondary metabolites such as tannins, terpenoids, alkaloids, glycosides and phenols which have been found to have antimicrobial properties [2, 3, 4]. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. They are used as remedy against chronic eczema, chronic ulcers, chronic rheumatism, chronic nervous diseases, madness, cholera, amenorrhoea, piles and fistula etc. [5].

It has been estimated that 14-28% of higher plant species are used in medicinal purposes and that 74% of pharmacologically active plant derived components were discovered after following up on ethno botanical uses of the plant [6]. Medicinal plants are used by 80% of the world population as the only available medicines especially in developed and developing countries [7]. The use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. Indian Systems of Medicine are among the well-known global traditional systems of medicine. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems, such as Ayurveda [8]. More than 18,000 species of flowering plants found in India [9, 10] among these nearly 1,745 species are from the Indian Himalayan region, and many of these are found in Uttarakhand [11, 12]. Uttarakhand has rich varieties of herbs, medicinal and aromatic plant species. The Garhwal Himalaya is one of the richest floristic zones of India and contains more than 300 species of medicinal plants [13]. Uttarakhand has a great diversity of flora and fauna and has different kind of geographical condition and vast biodiversity ranging from the snow bound peaks of the Himalayas with the highest Nanda Devi (7817m) to the sub-tropical Terai region [13-14].

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [15]. A great number of aromatic, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems [16]. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re- antioxidants in reducing oxidative stress-

induced tissue injury [17]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective [18]. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions [19]. The study done on medicinal plants strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [20]. On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied three medicinal plants *i.e*. *Cassia fistula* L., *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. and *Azadirachta indica* A. Juss. The free radical scavenging activity and total phenolic content was evaluated during the course of work. The chlorophyll content, carotenoids and total protein content were determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

2. Materials and methods

All selected medicinal plant species were collected from, FRI (Forest Research Institute) Dehradun, India and their characteristics presented in Table 1.

Biochemical Activities

The chlorophyll contents were estimated by using Dimethyl Sulphoxide (DMSO) spectrophotometric method [21] with optical density (OD) at 663 and 645 nm. Carotenoid content determined by the spectrophotometer method [22] at OD of 450 nm. The total phenolic contents were measured by using Folin-Ciocalteu spectrophotometric method [23]. Quantification was done with respect to the standard curve of gallic acid. The results were expressed in gallic acid equivalents (GAE) mg GAE/gm of the dry weight and total proteins estimated by using the spectrophotometric method.

Antioxidant Activities

The total antioxidant activity was measured by the DPPH radical scavenging assay method [24]. The radical scavenging activity of plant extracts against stable DPPH radical (DPPH*) was determined. Various amounts of extracts were mixed with 5 mL of 0.004% ethanolic solution of DPPH. Each mixture was kept for 30 min in the dark and the absorbance of the samples was read at 517nm using UV-spectrophotometer. A lower absorbance indicates higher radical scavenging power. DPPH radical scavenging activity was calculated by following equation.

$$\text{DPPH Radical scavenging activity (\%)} = [1- \frac{A_t}{A_o} \times 100]$$

Where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 517 nm.

Statistical analysis

The data presented in mean values ± SE. Measurements were performed on three replicates (n = 3) for each treatment. The differences between the means were compared using least significant differences at $p < 0.05$.

3. Results

All parameters in all three selected medicinal plants have been demonstrated in figure-1. Maximum (4.82±0.52) and minimum (3.51±0.55) *Chl a* content recorded in *Azadirachta indica* and *Cassia fistula* respectively. The range of *Chl a* from *Azadirachta indica* (4.82±0.52) > *Terminalia arjuna* (4.41±0.76) and > *Cassia fistula* (3.51±0.55). For *Chl b* contents maximum (2.47±0.41) recorded in *Terminalia arjuna* and minimum (1.98±0.22) in *Cassia fistula*. The *Chl b* content ranges from *Terminalia arjuna* (2.47±0.41) >*Azadirachta indica* (2.35±0.21) > and *Cassia fistula* (1.98±0.22). For total *Chl* measurements, maximum (7.17±0.85) found in *Azadirachta indica* followed by *Terminalia arjuna* (6.88±0.77) and *Cassia fistula* (5.49±0.57) (Fig 1A; Table 2). For carotenoid content measurements maximum (7.99±1.2) recorded in *Cassia fistula* followed by *Azadirachta indica* (4.96±0.56) and *Terminalia arjuna* (2.44±0.47) (Fig 1B; Table 2). The phenolic content measurements expressed in gallic acid equivalents (GAE), maximum recorded in *Terminalia arjuna* (35.23±2.01 to 70.82±0.50) followed by *Azadirachta indica* (39.93±1.23 to 55.67±1.89) and *Cassia fistula* (20.85±0.54 to 38.19±1.45) (Fig 1C; Table-3). Total protein content, maximum recorded in *Cassia fistula* (8.25±1.33) followed by *Azadirachta indica* (5.18±0.89) and *Terminalia arjuna* (4.66±0.55) (Fig D; Table 2). The maximum DPPH activity was recorded in *Terminalia arjuna* (76.55±3.2 to 93.56±3.23) followed by *Azadirachta indica* (70.23±2.3 to 81.50±2.98) and *Cassia fistula* (61.22±2.3 to 74.34±3.33) (Fig 1E; Table 3). The free radical scavenging action of ethanolic extracts of plant are in the order as *Terminalia arjuna* > *Azadirachta indica* > *Cassia fistula*. The extracts, which showed the strongest DPPH radical scavenging activity, is *Terminalia arjuna*, while the others show moderate antioxidant properties.

Table 1: Characteristics and medicinal properties of selected medicinal plants

Selected Plants	Common name	Family	Chemical constituents	Medicinal uses
Cassia fistula L.	Amaltus, Golden shower, Indian laburnum	Cisalpineaceae	Anthraquinones, Flavanoids, Flavan-3-ols, antioxidant, glucoside	Anti-Tumour, acidity, antioxidant, skin disorder, constipation, etc.
Azadirachta indica A. Juss.	Neem	Meliaceae	Nimbidin, nimbin, nimbidol & bakayanin	Wonder for skin, nail and hair problems, blood purifier, cure asthma, treat wound &rashes, muscle and join pain reliever, control diabetes, etc.
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Arjun tree	Combretacea	Arjunin	Cancer, dermatological, heart diseases & urinary disorders &hypertension.

Table 2: Biochemical analysis of selected medicinal plants

Test	*Cassia fistula*	*Terminalia arjuna*	*Azadirachta indica*
Chlorophyll a (*Chl a*)	3.51 ± 0.55	4.41 ± 0.76	4.82 ± 0.52
Chlorophyll b (*Chl b*)	1.98 ± 0.22	2.47 ± 0.41	2.35 ± 0.21
Total Chlorophyll	5.49 ± 0.57	6.88 ± 0.77	7.17 ± 0.85
Carotenoid	7.99 ± 1.20	2.44 ± 0.47	4.96 ± 0.56
Total Protein Content	8.25 ± 1.33	4.66 ± 0.55	5.18 ± 0.89

All parameters based on three replicates (n = 3). Significantly data were take on SD ($P < 0.05$).

Table 3: DPPH (2, 2'-Diphenyl picryl hydrazyl) radical scavenging activity (%) and Total Phenolic content in selected plants

Plant Species	% Radical Scavenging					Phenol (µg/mg GAE)				
	Conc. (µg/ml)					Conc. (µg/ml)				
	20	40	60	80	100	20	40	60	80	100
Cassia fistula	61.22±2.3	65.97±2.47	68.23±2.78	71.22±2.89	74.34±3.33	20.85±0.54	24.63±1.01	29.15±1.80	32.83±1.66	38.19±1.45
Terminalia arjuna	76.55±3.2	81.55±3.45	87.67±3.68	90.62±3.33	93.56±3.23	35.23±2.01	37.88±1.81	65.51±0.92	68.12±0.88	70.82±0.50
Azadirachta indica	70.23±2.3	71.05±2.32	74.23±2.78	75.63±2.34	81.50±2.98	39.93±1.23	40.81±0.67	48.99±1.06	50.28±1.11	55.67±1.89

All parameters based on three replicates (n = 3). Significantly data were take on SD ($P < 0.05$).

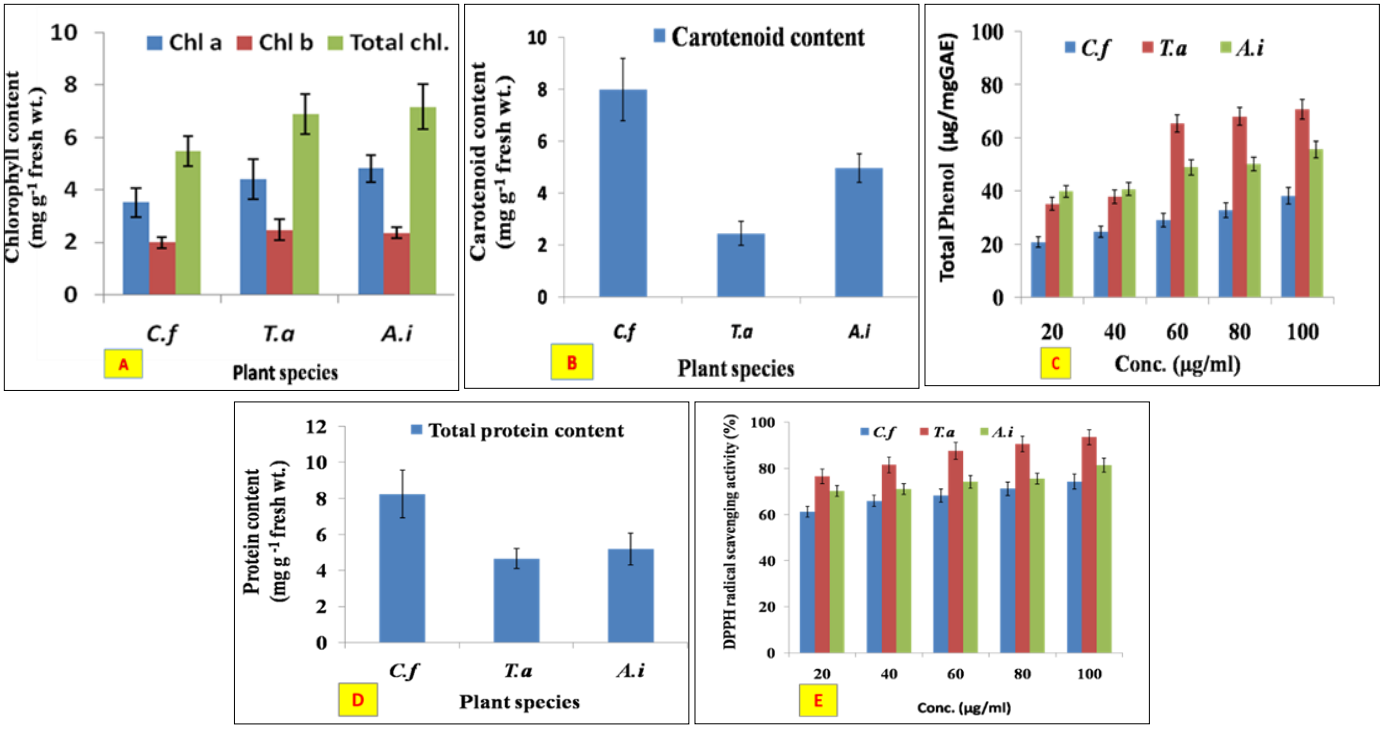

Fig 1: (A) Chlorophyll content, (B) Carotenoid, (D) Total Protein content, (E) DPPH and (C) Total Phenol content in different medicinal plants (*C.f = Cassia fistula, T.a =Terminalia arjuna, A.i = Azadirachta indica*)

4. Discussions

Chlorophylls are the green pigments that act as the principal photoreceptor molecules of plants. They are capable of absorbing certain wavelengths of visible light and then convert them into chemical energy. Two different forms of chlorophyll which are present in higher plants they are *Chl a* and *Chl* b [25]. Several studied proved that carotenoids have a positive role on the epithelization process and influence the cell cycle progression of the fibroblasts [26]. Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer [27]. In plants, carotenoids play a crucial role in protecting chlorophyll with antioxidant activity and these properties are one the many reasons that carotenoids are of interest to humans. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties [28]. Natural flavonoids have polyphenolic compounds which are mainly occur in plants as sugar derivative; glycoside [29-32]. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of these diseases. Free radicals scavenging assay is most important method for antioxidant measurement [29,33]. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful [34].

In this study, a significant linear correlation was found between the concentration of phenolic compounds and the antioxidant activity of extracts from different selected

medicinal plants. Among the three medicinal plants investigated we found that *Terminalia arjuna* exhibited maximum antioxidant activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

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