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IN VITRO–INVESTIGATIONS OF ANTIOXIDANTS & PHYTOCHEMICAL ACTIVITIES OF AQUEOUS EXTRACTS OF *TERMINALIA BELERICA* & *TERMINALIA CHEBULA*

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ABSTRACT

This study investigates the influence of extraction system on the extractability of phytochemical compounds and antioxidant activity of ethanolic extracts of *Terminalia belerica* & *Terminalia chebula.* It can be concluded that the solvent used affects significantly the phytochemical content and the antioxidant activity of the extract and therefore it is recommended to use more than one extraction system for better assessment of the antioxidant activity of natural products. Several of the investigated herbs contain substantial amounts of free radical scavengers and can serve as a potential source of natural antioxidants for medicinal and commercial uses. The phytochemical screening of Aqueous extract of *T.belerica* showed the absence of alkaloids, terpenoids and glycosides. Whereas *Terminalia chebula* showed the absence of alkaloids, amino acids, terpenoids and glycosides. *Terminalia belerica* showed the presence of amino acids, whereas *Terminalia chebula* showed the absence of amino acids. The antioxidant screening of Aquous extract of both plants showed the presence of enzymatic antioxidants such as catalase, peroxidase and ascorbate oxidase and non- enzymatic antioxidant such as ascorbic acid.

INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the country. In India thousands of species are known to have medicinal values. The medicinal actions of plants are unique to a particular plant species, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [1]. Medicinal plants play an important role in supporting healthcare system in India. According to the World Health Organization (WHO), 80% of the rural population in developing countries utilizes locally available medicinal plants for their primary healthcare needs Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases.. From ancient times, herbs have been used in many areas, including nutrition, medicine, flavoring, beverages, cosmetics, etc. The ingestion of fresh fruit, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases [2]. The higher intake of plant foods correlates with lower risk of mortality from these diseases. Antioxidants are substances that are able to prevent or retard oxidation of lipids, proteins and DNA; and to protect the compounds or tissues from damage caused by oxygen or free radicals. Therefore, their health promoting effects reduce the risk of various diseases [3]. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases [4]. Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables [5]. As many antioxidants are supplied from the diet, attention has been paid to intake of the micronutrient antioxidants (vitamins A, C, and E, polyphenols and carotenoids) and to know how it may help to protect individuals from an oxidizing environment and/or inflammatory airway disease. Approximately 60 % of the commercially available anti-tumoral and antiinfective agents are of natural origin [6]. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs[7].

EXPERIMENTAL SECTION

Material and Methods

Collection of plant materials

The plant material used was the dried leaves of medicinal plants which were *Terminalia chebula* & *Terminalia belerica* collected from forest region of Paonta sahib and were identified by Botanical Survey of India Dehradun.

Extraction of plant material:

The plant material taken for the study was stored under refrigerated condition till use. The samples were prepared by extraction of plant material with ethanol solvent by Soxhlet apparatus. By evaporating on water bath a crude extract was obtained of medicinal plants.

Storage:

Plant extracts were stored at the temperature of 4^0C till use for investigation.

Phytochemical investigations:

Alkaloid was investigated according to procedure given by [8].

Saponin and Flavonoids: were investigated according to the procedure of [8].

Tannins: Tannins were investigated according to the procedure of [9].

Glycoside: Glycosides were investigated according to the procedure of [10].

Terpenoids: Terpenoids were investigated according to the procedure of [10].

Reducing sugar was investigated according to the procedure of [8].

Amino acids were investigated according to the procedure of [8].
Determination of antioxidant activity

Assay of Catalase activity: Catalase activity was assayed by the method of[11].

Assay of Peroxidase activity: The assay was carried out by the method of [12].

Assay of ascorbate oxidase activity: Assay of ascorbate oxidase activity was carried out according to the procedure of [13].

Quantification of vitamins: The determination of ascorbic acid was carried out by the procedure given by [14].

RESULTS AND DISCUSSION

Phytochemical screening of plant materials

The phytochemical screening of the plants studied showed the presence of Flavonoids, reducing sugars, saponins and tannins, *Terminalia belerica,* showed the absence of alkaloids, terpenoids and glycosides. Whereas *Terminalia chebula* showed the absence of alkaloids, amino acids, terpenoids and glycosides. *Terminalia belerica* showed the presence of amino acids, whereas *Terminalia chebula* showed the absence of amino acids. Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of

blood lipids. Thus these plants have been used to lower the blood lipid content. Qualitative analysis carried out on each plant extract showed the presence of phytochemical constituents and the results are summarized in Table 1.

Table1. Phytochemical analysis of *Terminalia belerica* & *Terminalia chebula.*

TEST	*Terminalia belerica*	*Terminalia chebula*
Alkaloids	Negative	Negative
Saponins	Positive	Positive
Tannins	Positive	Positive
Amino acids	Positive	Negative
Terpenoids	Negative	Negative
Reducing sugars	Positive	Positive
Glycosides	Negative	Negative
Flavonoids	Positive	Positive

Antioxidant Activity of aquous extracts of *Terminalia belerica & Terminalia chebula*:

Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in the plant extracts, the herbal extracts exhibited good sources of water soluble antioxidants, phenolic compounds and antimutagens. Herbal drinks are becoming popular especially among health-conscious consumers since these beverages are prepared from natural ingredients. Many investigators reported that some herbal drinks contain many compounds such as polyphenols, flavonoids, isoflavones and glucosinolates.

Table 2. Enzymatic Antioxidant Analysis in aquous extracts of *Terminalia belerica & Terminalia chebula*.

Samples	Catalase µ/moles of H_2O_2 decomposed /min/g extract	Peroxidase 1U/L	Ascorbate oxidase µ mole /ml
Aqueous extracts of *Terminalia belerica*	0.1	10.47×10^3	1.54
Aqueous extracts of *Terminalia chebula*	0.2	11.9×10^3	1.4
	1 unit = µ/moles of H_20_2 decomposed /min/g extract	1 unit = µmoles pyrogallol oxidized/ min	1 unit = 0.01 O.D change /min

Among the aquous extracts of both plants the highest activity of Catalase was observed in *Terminalia chebula* (0.20 units/mg protein) and lowest in *Terminalia belerica* (0.10 units/mg proteins). In plants, antioxidant enzymes namely catalase [15]. The Peroxidase activity was observed to be low in aquous extract of *Terminalia belerica* (10.47×10^3 units/mg protein), while the activity increased in aquous extract of *Terminalia chebula* (11.9×10^3). The ascorbate oxidase activity was highest in *Terminalia belerica* (1.54 units/mg protein), and lowest in *Terminalia chebula* (1.4 units/mg protein). The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity [16].. Ethanolic extract *A. lamarckii* and its sub-fractions exhibited a good reducing power. Leaves of this plant are useful for curing diabetes . Decoction of bark has been used as an emetic in India . Methanol extract of *Alangium salviifolium* flowers have shown to antibacterial activity against both gram-positive and gram-negative bacteria. Methanolic extract of root of *A. salviifolium* have shown analgesis and anti-inflammatory activities in albino mice [17].

Table 3. Non –Enzymatic Antioxidant Activity (Ascorbic acid)

Samples	Vitamin C (mg/g)
Aqueous extracts of *Terminalia belerica*	0.092
Aqueous extracts of *Terminalia chebula*	0.087

Vitamin C content was high in aqueous extract of *Terminalia belerica* (0.092 mg/ g tissue), whereas low in *Terminalia chebula*, it is (0.087 mg/ g tissue). Ascorbate has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells and shown to function as a reductant for many free radicals [18]. Oxidative damage to cellular components such as lipids and cell membranes by free radicals and other reactive oxygen species is believed to be associated with the development of a range of degenerative diseases including heart diseases, cancer, inflammation, arthritis, immune system decline, brain dysfunction. Blackberries are a good source of anthocyanins in which the anthocyanin contents were reported to be 67.4–230 mg/100 g fresh weight [19]. Both the Plants may play an important role in the prevention of human diseases related to oxidative damage.

CONCLUSION

The results of the present study revealed that the Aquous extracts of both plants i.e.*T.belerica* and *T.chebula* have antioxidant properties since these contains enzymatic and non - enzymatic antioxidants, these can be very effective against microbes causing various diseases. *In vitro* assessment of the antioxidant activity of ethanolic fractions of both these plants to scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and highly reactive hydroxyl radicals showed that the semi - pure compounds present in the fractions are useful potential source of antioxidants and can be used in the therapy of diseases like cancer, coronary heart disease, ageing and any other disease related to oxidative stress. These fractions being non-toxic showed significant antioxidant activity at scavenging free radicals. They also significantly scavenge hydroxyl radical which is known to cause cellular damage.

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REFERENCES

[1]Wink M. Introduction biochemistry, role & biotechnology of secondary products. In: M. Wink, Ed, Biochemistry of secondary products metabolism. CRC Press. Bocaroton, FL. 1999, Pp.1-16.

[2] Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease.Crit Rev Food Sci Nutr. 2004, 44, 275–295.

[3] Manach C, Scalbert A, Morand C, et al. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004, 79, 27–47.

[4] Odukoya, A., Ilori, O., Sofidiya, M., Aniunoh, O., Lawal, B., Tade, I. Antioxidant activity of Nigerian dietary spices. EJEAF Che. 2005, 4: 6, 1086-1093

[5] Jiao, Z., Liu, j., Wang, S.. Antioxidant Activities of Total Pigment Extract from Blackberries. Food Technol. 2005,43, 1, 97-102.

[6] Cragg GM, Newman DJ, Weiss RB.Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. Semin Oncol.1997, 24, 156–163.

[7]Shweta Saboo, Ritesh Tapadiya, 1S. S. Khadabadi and 2U. A. Deokate. *In vitro* antioxidant activity and total phenolic, flavonoid contents of the crude extracts of *Pterospermum acerifolium* wild leaves (Sterculiaceae). J. Chem. Pharm. Res., 2010, 2(3):417-423.

[8] Harborne, J.B. . Phytochemical methods, London. Chapman and Hall, Ltd.1973, Pp. 49-188.

[9] Trease, G.E., Evans, W.C. Pharma cognsy.11th Edn. Brailliar Tiridel Can. Macmillan Publishers. 1989.

[10] Siddiqui, A.A., Ali.Practical Pharmaceutical chemistry.1st ed. CBS Publishers and Distributors, New Delhi.1997, pp . 126-131.

[11] Sinha, A.K. Colorimetric assay of Catalase. Anal. Biochem.1972, 47, 389-394.

[12] Addy, S.K. and Goodman, R.N. Polyphenol oxidase and peroxidase in apple leaves inoculated with a virulent or an avirulent strain for Ervinia amylovora. Ind. Phytopath.1972, 25, 575-579.

[13] Vines, H.M. and Oberbacher, M.F. Response of oxidation and phosphorylation in citrus mitochondria to arsenate. Nature. 1965, 206, 319-320.

[14] Sadasivam, S. and Manickam, A. Vitamins. In: Biochemical methods, Eds. Sadasivam, S. and Manickam, A.2nd Edition, New Age International (P) Limited, New Delhi.1997, 185-186.

[15] Hertwig, B. Steb, P.and Feierabend, J. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. Plant physiol.1999, 100, 1547-1553

[16] Foyer, C. Ascorbic acid. In: Antioxidants in higher plants, EDS. Alscher, R.G. and Hess, J.L. CRC press, Boca Raton.1993, p. 111-134.

[17] Aruoma OI.. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc.1998, 75, 199–212.

[18]Rajesh Kumar and S. Hemalatha. *In-vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. J. Chem. Pharm. Res., 2011, 3(1):259-267.

[19] A Kumaran; J Karunakaran. *LWT*., 2006, 40, 344-352.