In vitro antioxidant activities of Asteraceae Plants

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Abstract

Anaphalis neelgerriana DC and *Cnicus wallichi* DC belonging to the family Asteraceae (Compositae) are important medicinal plants indigenous to Nilgiris. Since the related species *Anaphalis morrisonicola* and *Cnicus benedictus* were reported for its anti cancer activities,the above mentioned plants were screened for *Invitro* antioxidant activity. *Invitro* antioxidant studies were carried out by DPPH, Nitricoxide and Hydrogen peroxide methods for the aerial part extracts of the plants. Different extracts were obtained from the aerial parts of the whole plant by successive solvent extraction and cold maceration process and subjected for *Invitro* antioxidant activity studies. Among the extracts tested, the ethyl acetate extract of *Anaphalis neelgerriana* DC and *Cnicus wallchi* DC showed significant anti oxidant activity in all the above methods. The potent ethyl acetate extract should be tested for anti oxidant activity in animal models.

Key words: *Anaphalis neelgerriana* DC, *Cnicus wallchi* DC, *Invitro* studies, Anti oxidant activity.

Introduction

Anaphalis neelgerriana DC belonging to the family Asteraceae (Compositae) is also known as Nilgiris everlasting, Cud weed, Helichrysm, *Raktaskandana* (Sanskrit) Varnapata, and Nilgiris katplaster. This plant is indigenous to Himalayas, Palani and Nilgiris hills of South India. It is a very small white leafed shrub, characteristic of dry and exposed situations in Nilgiris. It is 3 to 4 feet in height, and broad with numerous ascending usually opposite branches, clothed below by the dead leaves. Stem is rough with corky leaves, bluish white ¼ inch coated with soft white cotton closely appressed to the surface with recurved margins. *Anaphalis neelgerriana* DC flower heads and the hairs are employed for stopping bleeding. Fresh leaves are applied to wounds and cuts in the form of plaster and used as antiseptic[1,2]. *Cnicus wallichi* DC belonging to the family Asteraceae (Compositae) is a sub shrub and erect that occurs usually in Pulney hills, Himalayas, Palani and Nilgiri hills of South India. According to the ethno botanical information, the aerial parts of the plant extracts used externally for ulcers and abscesses and as an anti scorbutic used in the treatment of cuts, wounds and also as a galactogogue[3]. The roots have cooling effect and are used as a tonic and expectorant. In Nepal, a teaspoonful of pounded roots of the herb is given twice a day to control stomach inflammation[4]. The whole plant has a recognized medicinal value.This plant is commonly known as Indian thistle, *Cirsium wallichi*, thistle, buch bucham and Dharabindhak. Sesquiterpenes, flavonoids and steroids were reported as the main chemical constituents in *Cnicus wallichi* DC[5]. Since the related species *Anaphalis morrisonicola* and *Cnicus benedictus* were reported for its anti cancer activities, *Invitro* antioxidant activity screening studies by DPPH, Nitric oxide and Hydrogen peroxide methods was carried out for the aerial part extracts of these two medicinal plants.

Materials and Methods

The aerial parts of the *Anaphalis neelgerriana* DC and *Cnicus wallichi* DC were collected, identified and authenticated by Dr. S. Rajan, Botanist, Survey of medicinal plants and collection unit, Government Arts college, Ooty, Tamilnadu, South India. The specimen of the plant No.2526 and 2527 was preserved in the laboratory of TIFAC

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CORE HD, JSS college of Pharmacy, Ooty for further reference. The aerial parts of the plants were separately chopped in to small pieces and dried in shade. Then they were passed through sieve no 20 and used for extraction. The dried aerial parts of the plant material (500g) were extracted in a soxhlet apparatus by successive solvent extraction process. Petroleumether, chloroform, ethyl acetate and successive methanol extracts were obtained. 50% methanol, methanol and aqueous extracts were obtained by cold maceration[6]. The plant extracts were subjected to Preliminary phytochemical screening for the detection of various plant constituents present, according to standard procedures[7].

***In vitro* anti oxidant studies**

All the aerial part extracts of the *Anaphalis neelgerriana* DC and *Cnicus wallichi* DC were tested for *invitro* anti oxidant activity using standard methods. In all these methods a particular concentration ranging from 1000µg/ml to 0.45 µg/ml of the standard solution was used after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standard but without the reagents. A control test was performed without the extracts or standards. Percentage scavenging and IC_{50} values, Standard Error Mean (S.E.M), (Concentration of the sample required to inhibit 50% of radical IC_{50} value) were calculated and recorded in Table 1 and 2.

DPPH Radical scavenging activity assay

The anti oxidant activity of the plant extracts and pure compounds were assessed on the basis of radical scavenging effect on the stable DPPH (2,2 diphenyl 1 picryl hydrazyl) free radical . The assay was carried out in a microtitre plate. To 200ml of DPPH solution 10ml of each of the test sample or the standard solution was added separately in a microtitre plate. The final concentration of the test and standard solution used were 1, 0.5, 0.25, 0.125, 0.0625, 0.032, 0.016, 0.008, 0.004, 0.002, 0.001 mg/ml. The plates were incubated at 37^0C for 20 minutes and the absorbance of each solution was measured at 490nm using microtitre plate reader (ELISA) against the corresponding test and standard blanks. The IC_{50} values (the concentration of the sample required to scavenge 50% DPPH free radicals) were calculated [8].Results are tabulated in Table 1 and 2.

Nitric oxide radical scavenging activity assay

The reaction mixture (6ml) containing sodium nitro prusside (10mM, 4ml), Phosphate buffer saline (1ml) and the extract and standard solutions (1ml) were incubated at 25^0C for 150 minutes. After incubation 0.5ml of the reaction mixture containing nitrite was removed, 1ml of sulphanilic acid reagent 10.33% in 20% glacial acetic acid was mixed well and allowed to stand for 5 minutes for completing diazotization. Then 1ml of Naphthyl ethylene diamine dihydro chloride was added, mixed and allowed to stand for 30 minutes in diffused light. A pink coloured chromopore was formed. The absorbance of these solutions was measured at 540nm against the corresponding blank solution in 96 well microtitre plates using Elisa reader[9]. Results are tabulated in Table 1 and 2.

Hydrogen peroxide scavenging activity assay

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS at pH 7.4). Various concentrations of the extracts and standards in methanol (1ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 minutes, the absorbance was measured at 230 nm, against blank solution that contained extracts in PBS without hydrogen peroxide[10]. Results are tabulated in Table 1 and 2.

Results

All the results obtained are mentioned in Table 1 and 2. The preliminary phytochemical analysis of the aerial part extracts of the plants showed the presence of steroids, triterpenoids, phenolic compounds, Flavanoids, tannins and carbohydrates. The ethyl acetate extracts of *Anaphalis neelgerriana* DC and *Cnicus wallichi* DC exhibited a significant *invitro* anti oxidant activity among the extracts tested on comparison with the standards Ascorbic acid and Rutin. The ethyl acetate extract of *Anaphalis neelgerriana* DC produced IC_{50} values of 14.02 ± 2.50, 210 ± 4.42, and 59.38 ± 2.23 respectively. The ethyl acetate extract of *Cnicus wallichi* DC produced IC_{50} values of 12.48 ± 2.50, 210 ± 4.42 and 102 ± 2.23 respectively in DPPH, Nitric oxide and hydrogen peroxide methods.

Table 1 *Invitro* anti oxidant studies on *Anaphalis neelgerriana* DC

Sl. No	Extracts	Anti oxidant activity IC_{50} Mean + SE (μgm/ml)		
		DPPH	Nitric oxide	Hydrogen peroxide
1.	Petroleum ether	106 ± 3.20	800	212 ± 3.15
2.	Chloroform	85 ± 4.21	>1000	75 ± 1.12
3.	Ethyl acetate	14.02 ± 2.50	210 ± 4.42	59.38 ± 2.23
4.	Successive methanol	14.82 ± 2.28	285 ± 5.55	118.75 ± 4.15
5.	50% methanol	14.03 ± 1.27	295 ± 5.24	102 ± 2.25
6.	Aqueous	90.75 ± 2.23	310 ± 5.23	425 ± 3.11
7.	Methanol	28.12 ± 1.18	305 ± 5.15	325 ± 3.25
8.	Rutin	14.90 ± 0.55	270.90 ± 5.99	187.33 ± 3.93
9.	Ascorbic acid	18.73 ± 0.17	255.4 ± 5.56	125.20 ± 2.85

- **Average of three determinations**

Table 2 *Invitro* anti oxidant studies on *Cnicus wallchi* DC

Sl. No	Extracts	Anti oxidant activity IC_{50} Mean + SE (μgm/ml)		
		DPPH	Nitric oxide	Hydrogen peroxide
1	Petroleum ether	193.50 ± 3.50	>1000	212 ± 3.15
2	Chloroform	75 ± 4.21	375 ± 9.84	129 ± 1.12
3	Ethyl acetate	12.48 ± 2.50	210 ± 4.42	102 ± 2.23
4	Successive methanol	17.46 ± 2.28	285 ± 5.55	118.75 ± 4.15
5	50% methanol	23.11 ± 4.12	295 ± 5.24	120 ± 2.25
6	Aqueous	90.75 ± 2.23	310 ± 5.23	375 ± 3.11
7	Methanol	37.50 ± 1.18	305 ± 5.15	325 ± 3.25
8	Rutin	14.90 ± 0.55	270.90 ± 5.99	187.33 ± 3.93
9	Ascorbic acid	18.73 ± 0.17	255.4 ± 5.56	125.20 ± 2.85

***Average of three determinations**

Discussion

Free radicals are chemical species possessing an unpaired electron that can be considered as fragment of molecules and which are generally extremely reactive and short lived. They are produced continuously in cells as accidental by products of metabolism**.** There is increasing evidence to support the involvement of free radical reactions in several human diseases. In recent years it has become increasingly apparent that free radicals play a role in a variety of normal regulatory system, the de- regulation of which may play an important role in cancer.Active oxygen species and other free radicals have long been known to be mutogenic. Further, these agents have recently emerged as mediators of phenotypic and genotypic changes that lead from mutations to neoplasia. Free radicals may, therefore, contribute widely to cancer development in humans. In the last decade, evidence has accumulated that the free radical process, known as lipid peroxidation, plays a crucial and causative role in the pathogenesis of atherosclerosis, cancers, myocardial infraction and also in aging.Further, in recent years, increasing experimental and clinical data have provided compelling evidence for the involvement of oxygen free radicals in the three main disorders, namely, chronic lung disease, cancer and intra venticular haemorrhage. The theoretical basis of free radical involvement in these disorders is that oxygen centered radical and related reactive oxygen metabolites are found to be rapidly detoxified by the antioxidant defense mechanisms in specific tissues.

Preliminary phytochemical analysis revealed the presence of Flavanoids in the ethylacetate extracts of these two plants.The ethylacetate extract exhibited a significant antioxidant activity in all the methods carried out .Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. There is evidence that flavonoids are having antioxidant activities and having strong inhibition of lipid peroxidation *in vitro* and *in vivo.* Flavonoids may directly scavenge some radical species by acting as chain breaking antioxidants or they may recycle other chain-breaking antioxidants such as tocopherols by donating a hydrogen atom to tocopherol radical. Transition metals such as ferric and copper are important pro-oxidants and some flavonoids can chelate divalent metal ions, thus preventing free radical formation.

Conclusion

The ethyl acetate extracts of *Anaphalis neelgerriana* DC and *Cnicus wallichi* DC exhibited a significant *invitro* anti oxidant activity among the extracts tested in DPPH, Nitric oxide, hydrogen peroxide and hydroxyl radical scavenging by P-NDA methods. Preliminary phytochemical studies revealed the presence of flavanoids and their glycosides in ethyl acetate extract. The flavanoids are known for their potent antioxidant activity. Hence, the potent ethyl acetate extract should be tested for anti oxidant activity in (*in vivo*)animal models.

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