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# Phytochemical and Antioxidant Screening of the Aerial Parts of *Kyllinga nemoralis*



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## ABSTRACT

In recent years, the use of natural antioxidants present in the food and other materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Nutraceuticals are supposed to hold the key to a healthy society in the coming future. In the present study, an attempt has been made to evaluate the antioxidant activity of the aerial parts of the plant and results showed that the alcoholic extract of the plant were found to reduce ferric ions in a concentration dependent manner showing potent antioxidant activity of the plant.



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## INTRODUCTION

A close look at the ongoing research and investment into medicinal plants candidly points out that herbal therapeutics will continue to play an important role in human health. They are indicated in both prophylactic and curative treatments throughout the world. Though there is anecdotal evidence supporting the use of herbal medicines, scientific studies in this field are in their infancy.

Antioxidants are gaining a lot of importance as a panacea for a large number of lifestyle diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases, owing to sedentary way of life and stressful existence. Naturally, there is a dynamic balance between the amount of free radical produced in the body and antioxidants to scavenge them to protect the body against deleterious effects. The amount of antioxidant principles present under physiological conditions may be insufficient to neutralise free radical generated. Hence there has been an increased interest in preventive medicine in the development of natural antioxidants from plant materials. That is why plants with antioxidant properties are becoming more and more popular all over the world. [1,2,3]

*Kyllinga nemoralis* is widely distributed in tropical and subtropical area of the world. In India, it is distributed throughout the southern part. It is a terrestrial, perennial herb with white coloured flowers and fibrous roots [4]. As per ayurvedic medicine, the plant is used in treating fever, diarrhoea, cough, bronchitis and fistula. The plant is also reported to be a hepatoprotectant [5], anthelmintic [6] and anti-malarial agent [7].

## MATERIALS AND METHODS

### Plant material

The aerial parts of the *Kyllinga nemoralis* were collected from the campus of Pariyaram medical college hospital, Kannur, Kerala during the month of October 2013. Its botanical identity was confirmed by Dr. Radhika, Professor, Government Ayurveda College, Pariyaram, Kannur, Kerala and a voucher specimen (No.Ph.Cognosy/04/2014APSC) has been deposited at the Department of Pharmacognosy and Phytochemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.



**Fig 1. *Kyllinga nemoralis* showing white flowers**

**Successive solvent extraction [8,9]**

*Petroleum ether extract:* The coarsely powdered dried aerial parts of *Kyllinga nemoralis* was extracted with petroleum ether by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

*Benzene extract:* The marc left after petroleum ether extraction was dried and extracted with benzene by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

*Chloroform extract:* The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

*Acetone extract:* The marc left after chloroform extraction was dried and extracted with acetone by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

*Ethanollic extract:* The marc left after acetone extraction was dried and extracted with (95%) ethanol by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and concentrated.

*Aqueous extract:* The marc left after ethanol extraction was dried and extracted with distilled water by maceration process for 7 days. After completion of extraction, the solvent was removed by evaporation and concentrated.

The above extracts were used for the phytochemical analysis. The extractive values for each extract were calculated and recorded.

### **Phytochemical analysis of different extracts of *Kyllinga nemoralis* [8,9]**

#### **(a) Test for carbohydrates**

*Molisch's Test:*-To the extract 2 to 3 ml, add few drops of  $\alpha$ -Naphthol solution in alcohol, add concentrated sulphuric acid from the sides of the test tube.

*Fehling's Test:*-Mix 1ml of Fehling's A and 1ml Fehling's B in the test tube. Add equal volume of extract. Heat in the boiling water bath for 5-10 minutes.

*Benedict's Test:*-Mix equal volume of Benedict's reagent and extract in the test tube. Heat in a boiling water bath.

*Barfoed's Test:*-Mix equal volume of Barfoed's reagent and extract. Heat for 1-2 minutes in boiling water bath and cool.

*Seliwanoff's Test:*-Heat 3ml of Seliwanoff's reagent with 1ml of extract in water bath for 1-2 minutes.

*Tollen's Phloroglucinol Test:*- Mix 2.5 ml concentrated hydrochloric acid and 4 ml 0.5% Phloroglucinol. Add 1-2 ml extract. Heat it.

**(b) Test for proteins**

*Biuret Test*:-To 3ml of extract add 4% sodium hydroxide and few drops of 1% copper sulphate solution.

*Millon's test*:-Mix the extract with Millon's Reagent

**(c) Test for amino acids**

*Ninhydrin test*:-Heat 3ml extract and 3 drops of Ninhydrin solution in boiling water bath for 10 minutes.

**(d) Test for fats and oil**

*Solubility Test*: -Check the solubility in Ether, Chloroform, Benzene, Water and Alcohol.

*Saponification test*:-Evaporate the extract to get 10 ml oil. To oil add 25 ml 10% sodium hydroxide. Boil in boiling water bath for 30 minutes. Cool and add excess of sodium sulphate solution. Soap forms and rise to the top. Filter and to the filtrate add sulphuric acid. Evaporate, collect the residue, it contains glycerol. Dissolve the residue in ethanol. To the ethanolic solution, add few drops of potassium bisulphate. Heat vigorously and in other test tube add few drops of copper sulphate and sodium hydroxide solutions.

**(e) Test for steroids**

*Salkowski reaction*:-To the 2ml of extract, add 2 ml chloroform and 2 ml concentrated sulphuric acid through the sides of the test tube. Shake well.

*Liebermann - Burchard reaction*:- Mix 2 ml of the extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of concentrated sulphuric acid through the sides of the test tube.

*Liebermann reaction*:-Mix 3ml of the extract with 3ml acetic anhydride. Heat and add few drops of concentrated sulphuric acid through the sides of the test tube.

**(f) Test for volatile oils**

Hydro distill the drug material. Separate the volatile oil from distillate and check the odor of the oil, its solubility and also place a drop of oil in the filter paper to check the presence of greasy film on the paper.

**(g) Test for phenolic compounds**

To the extract add Ferric chloride, to the extract add dilute Iodine solution, to the extract add dilute ammonium hydroxide and potassium ferricyanide solution.

**(h) Test for flavonoids**

*Shinoda test*:-To the extract, add 0.5 g of magnesium turnings and few drops of concentrated hydrochloric acid from the sides of the test tube.

**(i) Test for alkaloids**

To the extract add dilute hydrochloric acid. Shake well and filter. To the filtrate perform the following tests.

*Dragendroff's test*: To the filtrate add few drops of Dragendroff's reagent.

*Mayer's test*:-To the filtrate add few drops of Mayer's reagent.

*Hager's test*:-To the filtrate add few drops of Hager's reagent.

*Wagner's test*:-To the filtrate add few drops of Wagner's reagent.

*Murexide test*:-To the extract add 3-4 drops of concentrated nitric acid. Evaporate to dryness. Cool and add 2 drops of ammonium hydroxide.

**(j) Test for glycosides**

**(i) Cardiac Glycosides**

*Baljet test*:- Sodium picrate is added to the thick section of the drug.

*Legal's test*:-To the extract, add 1 ml pyridine and 1 ml sodium nitroprusside

*Keller Kiliani test*:-To 2ml extract, add glacial acetic acid, one drop of ferric chloride and concentrated sulphuric acid.

(ii) Anthraquinone glycosides

*Borntrager's test*:-To the extract add dilute sulphuric acid. Boil and filter. To the cold filtrate, add equal volume of benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia.

(iii) C-glycosides

*Modified Borntrager's Test*:-To the extract add dilute sulphuric acid, ferric chloride and dilute hydrochloric acid. Heat for 5 min in boiling water bath. Cool and add any organic solvent. Shake well. Separate the organic layer. Add equal volume of dilute ammonia.

(iv) Saponin glycoside

*Foam test*:- Shake the drug powder or the extract vigorously with water.

*Haemolytic test*:- To the extract or drug powder add one drop of blood.

(v) Cyanogenetic glycosides

*Sodium picrate test*:- Soak a filter paper strip first in 10% acetic acid, then in 10% sodium carbonate and dry. In a conical flask place the moistened powdered drug. Cork it, place the above filter paper strip in the slit in cork.

### ***In vitro* Anti oxidant studies of *Kyllinga nemoralis* [10,11]**

Reduction of ferric ions by ortho-phenanthroline method:- Ortho substituted phenolic compounds are found more active than unsubstituted phenol. Hence, these compounds may exert pro-oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is measured at 510 nm. The reaction mixture consisting of 1 ml ortho-phenanthroline, 2 ml ferric chloride 200 mM and 2 ml of various concentrations of the extracts were incubated at ambient temperature for 10 min. The absorbance of the same was measured at

510 nm. The experiment was performed in triplicate. Percentage scavenging can be detected from the following formula;

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

## DISCUSSION

Free radicals are involved in the development and progression of many diseases. Highly reactive free radicals and oxygen species are present in the biological system oxidise nucleic acids, proteins, lipids and can initiate degenerative diseases. Scientific evidence showed that antioxidants reduce the risk of development of many chronic diseases. The main marker and characteristic of an antioxidant is its ability to trap free radicals. Most of the antioxidant compounds are derived from plant sources. Plants with antioxidant potential play an important role in protecting living organisms from the oxidative damage and act as lifestyle enhancers.

The aerial parts of *Kyllinga nemoralis* were subjected to successive solvent extraction with different solvents in the increasing order of polarity. Extracts were collected and subjected to phytochemical investigation and it showed the presence of glycosides, flavonoids, tannins, carbohydrates, triterpenoids, proteins, amino acids and phenolic compounds. Phenolic compounds have been reported to play a major role in reducing oxidative stress [12]. Ortho substituted phenolic compounds may exert pro-oxidant effects by interacting with Iron. Ortho phenanthroline quantitatively forms complexes with ferric ion which get disrupted in the presence of chelating agents. The extract interfered with the formation of ferrous ortho phenanthroline complex, thereby suggesting that the extract has metal chelating activity. The alcoholic extract of *Kyllinga nemoralis* and ascorbic acid were found to reduce ferric ions in a dose dependent manner. The significant inhibitory activity in the *in vitro* antioxidant studies of the extract with reduction of ferric ions indicates that free radical scavenging activity of the extract of the plant. Polyphenol rich diets provide significant protection against the development and progression of many chronic pathological conditions including cancer, diabetes, cardiovascular problems and aging. Although several biological effects based on epidemiological studies can be scientifically explained, the mechanism of action of some effects of polyphenols is

not fully understood. Further investigation can be carried out on fractionation, purification, identification of various active principles of the aerial parts of *Kyllinga nemoralis*.

## RESULTS

### Preliminary phytochemical screening

**Table 1. Successive solvent extraction of *Kyllinga nemoralis***

No	Solvent	Colour and consistency	Average extractive value (w/w)
1	Petroleum ether	Yellowish brown	1.453%
2	Benzene	Brownish black	1.211%
3	Chloroform	Greenish black	1.643%
4	Acetone	Greenish black	1.3785
5	Ethanol	Greenish black	4.213%
6	Water	Brownish black	6.131%

**Table 2. Qualitative chemical tests of *Kyllinga nemoralis***

No	Test	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloids	-	-	-	-	-	-
2	Carbohydrates	-	-	-	-	-	+
3	Phytosterols	+	+	-	-	-	-
4	Oils and Fats	+	+	-	-	-	-
5	Saponins	-	-	-	-	-	-
6	Phenolic compounds	-	-	-	-	+	+
7	Proteins and amino acids	-	-	-	-	-	+
8	Glycosides	-	-	-	-	+	+
9	Flavonoids	-	-	-	-	+	+

-absent, + present

Antioxidant studies

Table 3. *In vitro* Antioxidant studies of *Kyllinga nemoralis* by ortho-phenanthroline method

No	Concentration (Micrograms/ml)	Alcoholic (Ethanol) Extract		Ascorbic acid (Standard)	
		Absorbance	% scavenging	Absorbance	% scavenging
1	0.0	0.788		0.716	
2	2	1.108	28.88	1.856	58.39
3	4	1.121	29.70	1.888	58.46
4	8	1.124	29.89	1.904	62.39
5	16	1.139	30.81	1.979	63.82
6	32	1.151	31.53	1.996	64.12
7	64	1.198	34.22	2.432	70.55
8	128	1.214	35.09	2.889	75.21
9	256	1.642	52.00	3.412	79.01
10	512	1.762	55.27	3.899	81.63
11	1024	1.917	58.89	3.422	79.07

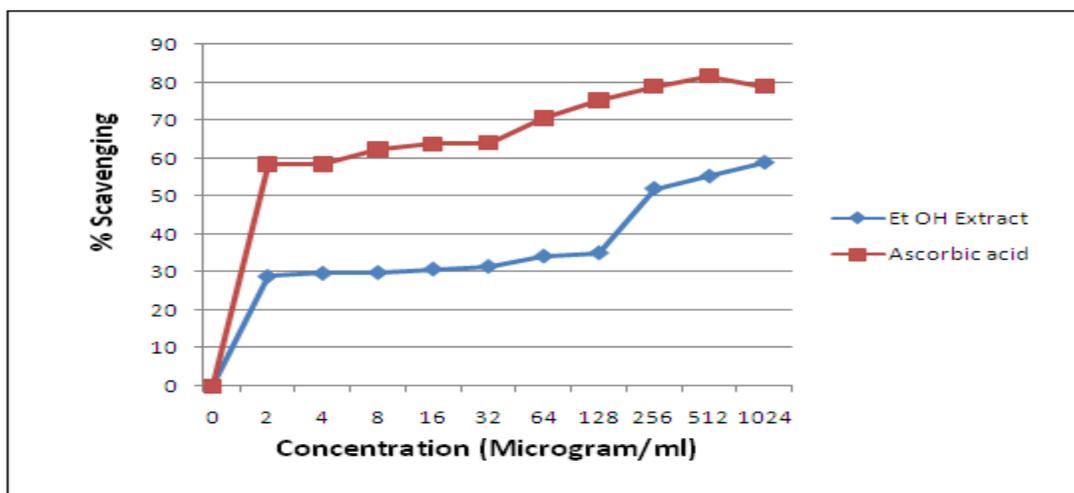


Fig 2. Antioxidant (*in vitro*) studies of *Kyllinga nemoralis* by ortho-phenanthroline method

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