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Research article

Evaluation of Antioxidant Activity of Some Medicinal plants and their Combination

Tanuj Joshi*, Vijay Juyal

ABSTRACT

Introduction: Oxidative Stress leads to several complications within the human body. It is the reason behind the generation of several diseases. Free radicals if generated in excess amount can damage the body to a great extent. Finding newer and potent medicinal plants that can fight oxidative stress can be useful in combating the harmful effects of free radicals. Methods: In the current study ethanolic extract of *Ocimum kilimandscharicum*, *Thymus serpyllum*, *Spilanthes acmella* and their combination in equal ratio were used for their ability to counter oxidative stress. The plants were collected from the district of Pithoragarh, Uttarakhand and extracted by soxhlet's apparataus using absolute ethanol (99.9%). The extracts were then dried and used for the study. Result: It was seen that highest absorbance was shown by ascorbic acid at the lowest as well as the highest concentration in the reducing power assay. Also, the combination of the extracts showed the highest absorbance among all the extracts at both the lowest and highest concentration. Conclusion: A higher absorbance indicates a better antioxidant potential. The best effect was shown by the combined extract among all the extracts.

Key words: Oxidative stress, Reducing power, Flavonoids, Phenols.

Tanuj Joshi*, Vijay Juyal

Department of Pharmaceutical Sciences, Bhimtal, Pin: 263136, Kumaun University (Nainital), INDIA.

Correspondence

Tanuj Joshi

Department of Pharmaceutical Sciences, Bhimtal, Pin: 263136, Kumaun University (Nainital), INDIA.

E-mail: joshitanuj18@gmail.com

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INTRODUCTION

Oxidative damage and antioxidants are assuming great interest in today's modern world. Cancer, cardiovascular diseases and other types of diseases may be due to the result of free radicals.[1] With newer insights into the fact that oxidative species can lead to a number of health problems and complications, finding treatment of oxidative damage and oxidative stress is acquiring greater significance. Phytochemicals obtained from plants are gaining popularity day by day in countering harmful effects of oxidative species and diseases associated with oxidative damage. Various fruits, vegetables and medicinal plants contain numerous phytochemicals.[1,2] Many phytochemicals have been isolated and tested for their therapeutic potentials but many phytochemicals are still there which need to be discovered in future. Phytochemicals have a wide variety of beneficial effects. They possess antioxidant activity, enhance immunity, reduce aggregation of platelets, influence the activity of hormones and have anticancer effects. Besides the above mentioned pharmacological activities, they produce numerous other actions as well.[3] Various phytochemicals have various pharmacological activities. Cellulose, lignins, gums and pectins bind toxins. Alkaloids, phenolic compounds and terpenoids have antimicrobial potential. Ascorbic acid, carotenoids, phenols and flavonoids have potent antioxidant capabilities. Antitumor activities have been shown by flavonoids, phenols etc. Carotenoids, tocopherols, phenols, flavonoids etc function as detoxifying agents.[2] Phenols and flavonoids are some plant constituents that have gained popularity over the years for treatment of oxidative stress. Many medicinal plants contain different phenolic compounds and flavonoids that help in a variety of diseases. In many experiments flavonoids and phenols have proved their potential in protecting the body against harmful effects of free radicals.[1,2] They protect proteins, lipids and deoxyribonucleic acid (DNA) against harmful effect of the free radicals. Also phenolic compounds and flavonoids may assist the natural antioxidant defence system of the body and help to fight oxidative damage within the body.[4] In the present study three medicinal plants *Ocimum kilimandscharicum* (OCM), *Thymus serpyllum* (THY), *Spilanthes acmella* (SPL) and their combination in 1:1:1 ratio (COMB) were assessed for their phytochemical constituents and antioxidant activity.

PLANT COLLECTION AND IDENTIFICATION

Aerial parts of *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella* were collected from the herbal gardens of Defence Institute of Bioenergy Research, Pithoragarh. The plant samples were preliminarily identified by ICAR-National Bureau of Plant Genetic Resources, Regional station, Niglat, Bhowali, Uttarakhand. The plant samples were further sent to Botanical Survey of India, Northern Botanical Survey of India for final authentication.

Preparation of the extract

After collection, the aerial parts of the above mentioned plants were dried. After drying the aerial parts were powdered and extracted with absolute ethanol (99.9%). Soxhlet's assembly was used for the extraction. The extract so obtained was then dried using rotatory vaccum flash evaporator.[5]

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Experimental procedure

Phytochemical screening of the extracts

The extracts of *Ocimum kilimandscharicum* (OCM), *Thymus serpyllum* (THY) and *Spilanthes acmella* (SPL) were individually subjected to phytochemical screening. Also, the extracts of the above mentioned plants were combined in equal ratio (1:1:1) and subjected to phytochemical screening according to procedures mentioned in literature.[6,7]

In-vitro antioxidant activity using reducing power assay

Seven log concentrations (0.0001-50 µg/ml) of the extracts namely OCM, THY, SPL and COMB (combination of *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella* in 1:1:1 ratio) in 1 ml of water were mixed with phosphate buffer (2.5 ml, 0.2 mol, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50° C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture. Centrifuged the mixture at 3000 × g for 10 min. Upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm (BMG FLUOSTAR, GERMANY).[8-10]

RESULTS

Phytochemical screening of the extracts

The individual extracts as well as the combined extracts were subjected to preliminary phytochemical screening tests using various methods. The results are given in the Table 1.

Reducing power assay

Individual extracts, combined extract and ascorbic acid showed different absorbance at different concentrations. A higher absorbance indicates higher reducing power due to formation of reduced intermediate (Table 2).

Table 1: Phytochemical screening of OCM, SPL, THY and COMB.

PHYTOCEMICAL TESTS	OBSERVATIONS	RESULTS OCM	RESULTS THY	RESULTS SPL	RESULTS COMB
Alkaloids					
(a) **Dragendorff's test**	Orange brown precipitate	Absent	Absent	Absent	Absent
(b) **Mayer's test**	Precipitate formation	Absent	Absent	Absent	Absent
(c) **Hager's test**	Yellow precipitate	Absent	Absent	Absent	Absent
(d) **Wagner's test**	Reddish brown precipitate	Absent	Absent	Absent	Absent
(e) **Tannic acid test**	Buff colored precipitate	Absent	Absent	Absent	Absent
Proteins					
(a) **Biuret test (General Test)**	Violet or Pink color	Absent	Absent	Absent	Absent
(b) **Millon's test (for protein's)**	White precipitate. Warm precipitate, it turns brick red or the precipitate dissolves giving red colored solution	Absent	Absent	Absent	Absent
Amino acids					
(a) **Ninhydrin test (General test)**	Purple or bluish color	Absent	Absent	Absent	Absent
Carbohydrates					
(a) **Molisch's test (General test)**	Violet ring is formed at the junction of two liquids	Present	Present	Present	Present
Saponin Glycosides					
(a) Foam test	Persistent stable foam was observed on shaking the extract with water	Present	Present	Present	Present
Flavonoids					
(a) Sulphuric Acid test (66% or 80%)	Deep yellow solution (flavones and flavonols)	Present	Present	Present	Present
(b) On addition of lead acetate to residue	Yellow colored precipitate	Present	Present	Present	Present
(c) Addition of increasing amount of sodium hydroxide to the residue	Yellow coloration, which decolorizes after addition of acid	Present	Present	Present	Present
Phenols and Tannins					
(a) 5% $FeCl_3$ solution	Deep blue-black color	Present	Present	Present	Present
(b) Lead acetate solution	White precipitate	Present	Present	Present	Present
(c) Dilute Potassium permanganate solution	Decoloration	Present	Present	Present	Present

Table 2: Absorbance values at various concentrations of different extracts and ascorbic acid in reducing power assay.

	Absorbance at various concentrations						
	0.0001 µg/ml	0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	1 µg/ml	10 µg/ml	50 µg/ml
OCM	0.21	0.24	0.23	0.9	0.95	1.41	1.64
THY	0.22	0.21	0.27	0.33	0.32	0.41	0.52
SPL	0.13	0.15	0.17	0.25	0.28	0.34	0.47
COMB	0.24	0.22	0.28	1.57	1.64	1.72	1.82
ASCORBIC ACID	0.42	0.97	1.35	1.66	2.18	2.22	2.71

DISCUSSION

In the present study it was seen that at the lowest concentration (0.0001µg/ml) the highest absorbance was shown by ascorbic acid and lowest absorbance was shown by SPL. At the highest concentration (50 µg/ml) highest absorbance was shown by ascorbic acid and lowest absorbance was shown by SPL. Highest absorbance was shown by THY in lowest concentration (0.0001µg/ml) and highest absorbance was shown by OCM at the highest concentration (50 µg/ml) among the individual extracts. Among all the extracts COMB showed better effect than the individual extracts in the reducing power assay at both the lowest and highest concentrations (Table 2). In reducing power assay antioxidants provide a beneficial effect by breaking the free radical chain by donating a hydrogen atom.[11] In this assay antioxidants act as reductants and reduce Fe^{3+}/ferricyanide complex to ferrous form. The absorbance of ferrous ions can be measured at 700 nm. In this assay increase in absorbance is an indicator of increasing reductive ability.[12]. Phytochemical screening has revealed the presence of antioxidant phytochemicals like, phenols and flavonoids in the extracts of the OCM, THY, SPL and COMB (Table 1). As has been discussed in the introduction that phenols and flavonoids have potent antioxidant activity, thus the presence of these phytochemicals might be responsible for the antioxidant activity of these extracts.

CONCLUSION

Diseases due to oxidative stress are posing a significant burden on humans. Newer research is being carried out by scientists to study the impact of free radicals in causing diseases. Medicinal plants have the potential to become frontline medicines in the treatment of oxidative stress. Also, these plants have the advantage that they are economical and a safe alternative to allopathic drugs and synthetic antioxidant formulations. Newer medicinal plants should be explored in detail so that we can come up with potent antioxidants. In the present study ascorbic acid, individual extracts of the three plants used in the study and the combination of these individual extracts were studied for antioxidant activity using reducing power assay. The results showed that even at very low concentrations the individual extracts and their combination demonstrated decent reductive ability in comparison to the standard antioxidant ascorbic acid. Also, the combination of the plant extracts used in the study showed the highest antioxidant potential in the present study when compared to the individual extracts. Thus the extracts of the plants used in the present study and their combination can prove to be good antioxidants if properly formulated and marketed.

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