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Evaluation of the Synergistic Effects on Antioxidant Activity of Different Combinations of Some Medicinal Plants Extracts

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Abstract

Many medicinal plants demonstrate significantly high antioxidant activity when used in combination than when used alone. However, the mechanism underlying this synergism is still poorly understood. This study aimed to investigate the synergistic antioxidant activity of methanolic extracts of Citrus medica, Azadirachta indica, Carissa carandas and Allium sativum in three combinations. The antioxidant activity of methanolic extracts were investigated using the 2,2-diphenyl-1-picrylhydazyl (DPPH) free radical -scavenging assay. Results, showed free radical scavenging activity of Citrus medica-182.08 µg/ml, Azadirachta indica-76.62 µg/ml, Carissa carandas-57.67 µg/mland Allium sativum -137.75 µg/mlwhereas, combinations of Citrus medica (Leaves) + Azadirachta indica (Leaves), Citrus medica (Leaves) + Carissa carandas (Leaves) and Allium sativum (Buds) + Carissa carandas (Leaves) showed IC_{50} value43.42 µg/ml, 35.00 µg/ml and 36.53 µg/ml respectively. The results of present study suggest that crude methanolic plant extracts were showing lowest antioxidant activity but when used in combinations, showed good synergistic antioxidant activity.

Keywords: Antioxidant activity, DPPH radical scavenging assay, Medicinal Plants, Synergistic effect

Introduction

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability[1]. Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries[2]. Natural antioxidants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory, and anti-aging properties[3,4,5,6]. Numerous vegetables, crops, spices and medicinal herbs have been tested in an effort to identify new and potentially useful antioxidants[7,8,9,10]. More recently, it has become evident that phenolic natural products may reduce oxidative stress by indirect antioxidant action.

Materials and Methods

Materials

The reagents and chemicals DPPH (2, 2-Diphenyl-1- picryl-hydrazyl), α-tochopherol (Sigma- Aldrich, Germany), L-Ascorbic acid (Central Drug House (P) Ltd, New Delhi), Naphthyl ethylenediamine dihydrochloriode(Central Drug House (P) Ltd, New Delhi) and Sodium nitroprusside (Merck), Methanol (Sisco Research Laboratories Pvt. Ltd) were used.

Preparation of crude plant extracts

The medicinal plants used in this study, were collected from local region of Agra and Mathura district of country. Plant material consisting of mature leaves of *Carissa carandas*(Karonda), *Citrus medica* (Nimbu), *Azadirachta indica* (Neem) and buds of Allium *sativum* (Garlic) were collected and shade dried for 3-4 days. The dried plant materials were powdered using grinder. The extraction was done at room temperature with methanol in soxhlet apparatus for up to 24 cycles. The methanolic extracts were evaporated to dryness in a vacuum rotary evaporator (Heidolph, Germany) at set bath and cooling temperature of 35°C and 4°C respectively along with 147 bar vacuum pressure. These methanolic extracts were used for analyse the antioxidant capacity.

Screening for antioxidant activity

DPPH radical scavenging activity

Antioxidant activity of plant extracts was evaluated based on the radical scavenging effect of the stable 2, 2-diphenyl-1-picryal-hydrazyl (DPPH) radical using modified method (11). The dilutions of the test extracts ranging 7.8-1000 µg/ml concentrations were prepared in methanol. Ascorbic acid was used as standard in 7.8-1000 µg/ml concentrations. A 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of sample solution at different concentrations (7.8-1000 µg/ml) and standard separately. These solution mixtures were kept in dark for 30 min. and optical density was measured at 517 nm. Methanol (1ml) with DPPH solution (0.1mM, 1ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below (12). IC_{50} values were calculated at different intervals for test samples and standard using Finney, 1962 (13).

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where A= optical density of the blank and B= optical density of the test sample.

Evaluation of the antioxidant activity of plant crude extracts in combination

Three different combinations of the extracts Table 1, 2 and 3 were tested by using DPPH radical scavenging method. The results of the combined effects of the extracts were categorized as synergism, additive, indifference, or antagonism. For the binary mixtures(A + B) experimental data were transformed to fractional inhibitory concentration (FIC) as:

$$FIC_A = \frac{\text{Activity of compound A in the presence of B}}{\text{Activity of compound A individually}}$$

$$FIC_B = \frac{\text{Activity of compound B in the presence of A}}{\text{Activity of compound B individually}}$$

Subsequently, to establish if the binary mixtures tested are synergistic, antagonistic or additive, the fractional inhibitory concentration index (FIC_{index}) was calculated as:

$$FIC_{index} = FIC_A + FIC_B$$

Data for doses points appearing below the additively line are considered as synergic effects in a range of $FIC_{index} < 0.9$, additive effects in a range $0.9 < FIC_{index} < 1.1$ and antagonic effects for $FIC_{index} > 1.1$(14).The test extracts were mixed in

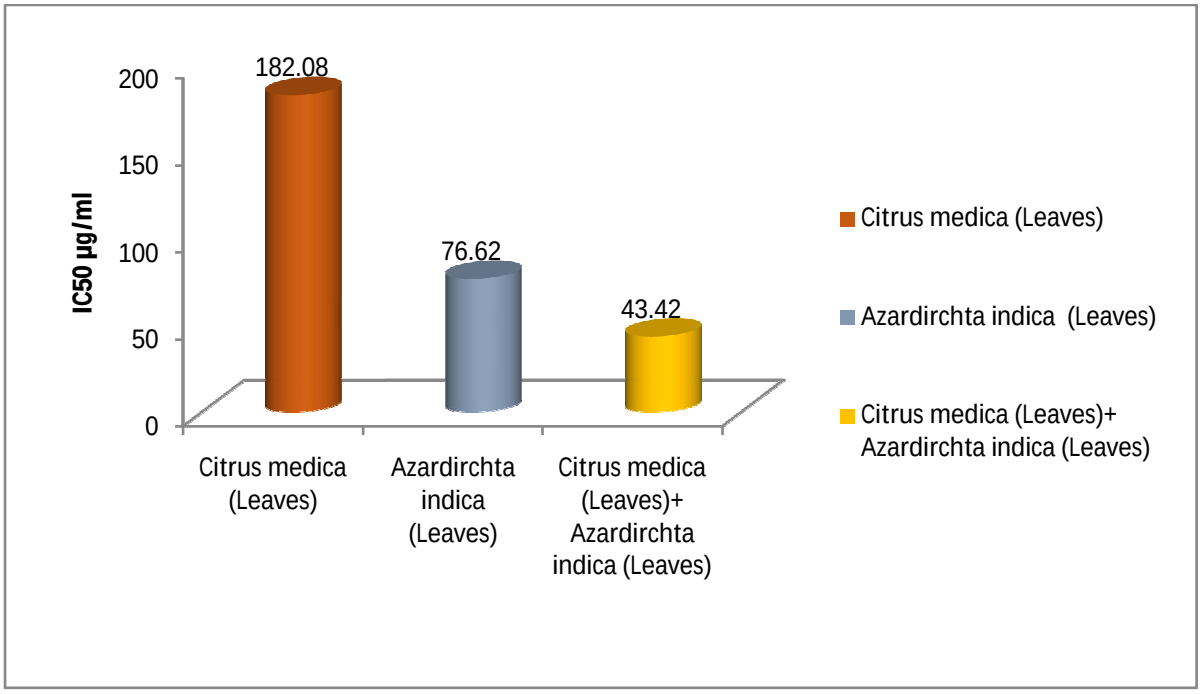
equal proportion of 1000 µl of each extract.

Results and Discussion

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants. The percentages of remaining DPPH in the presence of the methanolic extracts of plants separately and in combinations, at different concentrations are shown in Table 1, 2 and 3.

Table 1 Mean of percentage inhibition and IC_{50}values of *Citrus medica* (Leaves) and *Azadirachta indica* (Leaves) alone and in combination

Concentration of plant extracts →	7.8 µg/ml	15.6 µg/ml	31.2 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	IC_{50} µg/ml
***Citrus medica* (Leaves)**	8.5	11.9	25.5	30.9	44.7	49.7	72.9	75.5	**182.08**
***Azadirachta indica* (Leaves)**	11.2	18.0	30.3	56.6	68.4	77.3	78.2	78.9	**76.62**
***Citrus medica* (Leaves)+ *Azadirachta indica* (Leaves)**	16.7	28.1	49.6	58.7	66.7	86.6	88.9	90.0	**43.42**

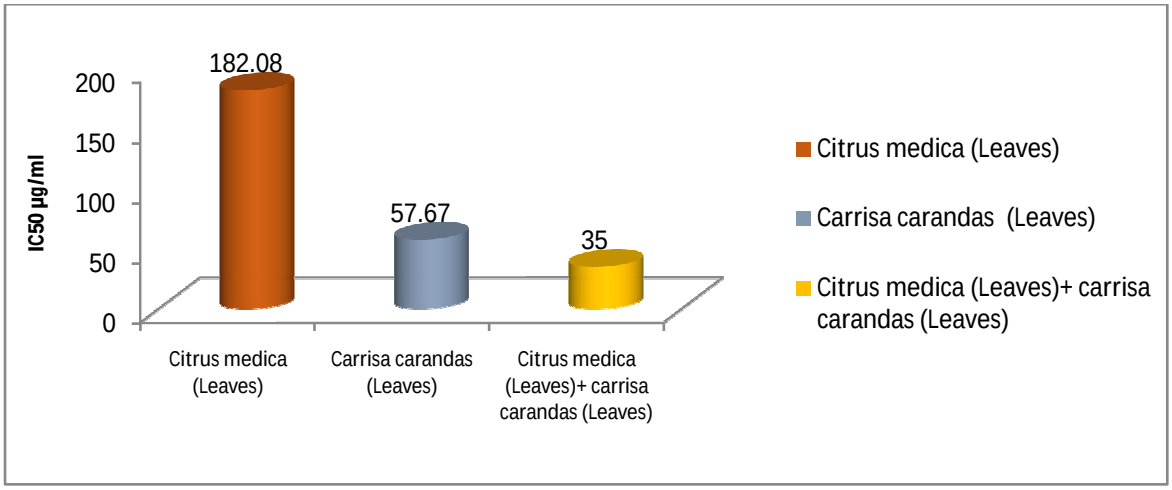


Graph 1- DPPH scavenging activity of *Citrus Medica and Azadirachta india* (leaves)seperately and in combination

As shown in Graph-1, methanolic extract of *Citrus medica* is showing very poor DPPH activity 182.08 µg/ml as well as weak *Azadirachta indica* shown also show poor value 76.62 µg/ml whereas, both plant leaves extracts used in combination they show synergistic effects (43.42 µg/ml).

Table2 Mean of percentage inhibition and IC_{50}values of *Citrus medica* (Leaves) and *Carrisa carandas* (Leaves) alone and in combination.

Concentration of plant extracts ⟶	7.8 µg/ml	15.6 µg/ml	31.2 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	IC_{50} µg/ml
***Citrus medica* (Leaves)**	8.5	11.9	25.5	30.9	44.7	49.7	72.9	75.5	**182.08**
***Carissa carandas* (Leaves)**	13.6	22.3	36.9	63.2	75.2	76.2	80.6	82.9	**57.67**
***Citrus medica* (Leaves +*Carissa carandas* (Leaves)**	17.6	29.8	47.3	75.3	80.3	82.7	86.2	92.2	**35.00**

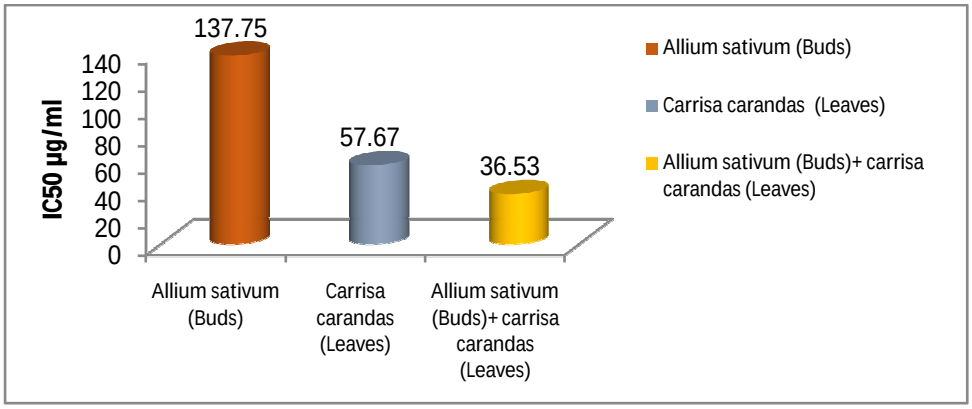


Graph 2- DPPH scavenging activity of *Citrus medica* and *Carrisa carandas* (leaves) seperately and in combination

Graph-2, also shows the synergistic effect (35 µg/ml) of *Citrus medica* and *Carrisa carandas* leaves extraxts in combination. When these both extracts used checked for antioxidant activity individually they show poor activity *Citrus medica* 182.08 µg/ml and *Carrisa carandus* 57.67 µg/ml.

Table 3 Mean of percentage inhibition and IC_{50}values of *Allium sativum* (Leaves) and *Carrissa carandas* (Leaves) alone and in combination.

Concentration of plant extracts ⟶	7.8 µg/ml	15.6 µg/ml	31.2 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	IC_{50} µg/ml
***Allium sativum*(Buds)**	9.2	15.2	21.2	32.9	49.5	61.8	76.5	81.1	**137.75**
***Carissa carandas* (Leaves)**	13.6	22.3	36.9	63.2	75.2	76.2	80.6	82.9	**57.67**
***Allium sativum*(Buds)+ *Carissa carandas* (Leaves)**	29.2	32.0	46.6	61.3	71.0	80.1	82.2	87.4	**36.53**



Graph 3- DPPH scavenging activity of *Allium sativum* (Buds) and *Carrisa carandas* (leaves) seperately and in combination

Allium sativum buds extract is showing poor antioxidant activity i.e. 137.75 µg/ml, Carissa carandas leaves extract also showing weak DPPH radical scavenging activity (57.67 µg/ml) but when these both extracts mixed and used for antioxidant activity, this combination showing good antioxidant activity 36.53 µg/ml comparatively (Graph-3).

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