Invitro Antioxidant Activity of Selected Antiasthmatic Herbal Constituents

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Abstract: *Asthmatic patients are more subjected to continuous oxidative stress. Boosting the antioxidant defenses of asthma patient could be beneficial as it may help thwart symptoms of the lung disease and asthma. Considering these facts different plant extracts and phytoconstituents with proven anti asthmatic property had been subjected to antioxidant study at various combinations using Diphenyl Picryl Hydrazyl radical scavenging method, Hydrogen Peroxide radical scavenging method, Nitric oxide radical inhibition method, ABTS radical scavenging method and Lipid peroxidation assay method.*

All the selected herbal based constituents and extracts at different combination showed a potent antioxidant activity by Nitric oxide radical inhibition activity method followed by ABTS method. The present finding suggests that the selected plant based anti asthmatic constituents namely Coleus extract (10% Forskolin), Piper longum extract (20% Piperine), CurcuminC3Complex (60% Curcumin), Vasaka (30% Vasicinone), Forskolin (95%), Piperine (98%), Curcumin C3 Complex (98.5%) and Vasicinone (75%) could be utilized in alternate anti asthmatic therapy, since they play a vital role in scavenging Nitric oxide which could prevent the bronchial inflammation in asthmatic patients.

Key words: *Anti oxidant, Anti asthma, Curcumin, Forskolin, Piperine.*

INTRODUCTION

Natural products and drugs as antioxidant have received much attention all over the world as an alternative solution to health problems and stress. An excess of oxidative stress can lead to the oxidation of lipids and proteins which is associated with changes in their structure and functions. When the body is exposed to air pollution, cigarette smoke, chemicals, toxic fumes, etc., electrons can be pulled out of orbit. The atom, which is missing the electron, becomes unstable and begins to grab electrons from other nearby molecules to replace it, causing chain reactions. The unstable electrons are called Free radicals [1].

"Free radicals" can also damage the cell membranes and intracellular structures such as the mitochondria, nucleus and DNA, causing mutations. Damaged cell membranes also allow viruses and bacteria to enter the body more freely and asthmatic patients are subjected to continuous oxidative stress associated with viral and bacterial infections [2].

Adhatoda vasica is an Indian herb widely used as a bronchodilator in traditional practices. Vasicinone, an oxidation product of vascinine is more potent bronchodialator[3]. The mechanism of pipernine's anti inflammatory activity may be due to its possible anti oxidant property. There are a few studies suggests that Piperine may inhibit lipid peroxidation[4]. Coleus is an effective smooth muscle relaxant, resulting in bronchodialation, increased vital capacity and increased forced expiratory volume[5]. Curcumin increases survival of cells exposed to the enzyme Xanthine oxidase, which stimulates super oxide and hydrogen peroxide production.[6]

Plant extracts and phytoconstituents with proven anti asthmatic property had been subjected to comparative antioxidant study individually and in various combinations with curcuminoids.

MATERIALS AN D METHODS:

1, 1-Dipheny1-2picryl hydrazyl (DPPH), 2, 2' Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), were obtained from Sigma Aldrich Co., St. Louis, USA. Phosphate Buffer Saline from Gibco-BRL. Rutin from Acros Organics., New Jersey, USA. Naphthyl Ethylene Diamine Dihydrochloride (NEDD) from Roch Light Ltd., Suffolk, UK. Ascorbic acid from SD Fine Chemicals Ltd., Mumbai, India. Sodium nitroprusside, Dimethyl sulphoxide, Potassium chloride and Sodium chloride from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acids, Sodium bicarbonate, Disodium hydrogen phosphate from E-Merck (India) Ltd., Mumbai, India.

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All chemicals used were of analytical grade. Forskolin (95%)Piperine (98%), Curcumin(98.5%), Vasicinone (75%), Standardized extracts containing Coleus Forskohli (10% Forskolin),Piper longum (20% Piperine),Curcumin C3 complex (60% curcuminoids), Adhatoda vasica (30% Vasicinone) were obtained from SAMI LABS Ltd Bangalore, along with spectral data to confirm its purity.

Diphenyl Picryl Hydrazyl (DPPH) radical Scavenging Method[7,8,9]

Test solutions were prepared by dissolving 21 mg of each of the extracts of Forskolin (95%), Piperine (98%), CurcuminC3complex (Curcumin, Desmethoxy Curcumin, Bisdesmethoxy Curcumin), Vasaka extract (30% Vasicinone), Coleus extract (10% Forskolin), Piper longum extract (20%Piperine) in distilled DMSO separately to obtain a solution of 21 mg/ml concentration. Each of these solutions was serially diluted separately to obtain the final concentration ranging from 1,000 g/ml to 0.9765 g/ml.

21mg of each of standard, ascorbic acid and rutin were prepared at the concentration of 21 mg/ml with DMSO and serially diluted to get lower concentrations. The assay was carried out in a 96 well microtitre plate. To 200l of DPPH solution, 10l of each of the test sample or the standard solution was added separately. The plates were incubated at 37^0C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Scavenging of Hydrogen peroxide radicals (H2O2)[9,10,11]

30 mg of each extracts and the standards (rutin and ascorbic acid) were dissolved in 10 ml of methanol and the solutions were serially diluted to obtain the lower concentration. A solution of H_2O_2 (20 mM) was prepared in PBS, (pH 7.4). Various concentrations of 1 ml of the extracts and standards in methanol were added to 2 ml of H_2O_2. The absorbance was measured at 230 nm after 10 min against a blank solution that contained extracts in PBS without H_2O_2.

Nitric oxide radical inhibition activity[9,11]

42 mg of ascorbic acid and rutin were separately dissolved in 2ml of DMSO to get 21mg/ml concentration and serially diluted to get lower concentrations. The reaction mixture containing 4ml of SNP (10 mM), 1ml of PBS and 1ml of extract in DMSO were incubated at 25^oC for 150 minutes. After incubation 0.5 ml of the reaction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution. Ic_{50} value obtained is the concentration of the sample required to inhibit 50 % nitric oxide radical.

ABTS radical scavenging method[9,12,13]

Solution I- ABTS solution (2 mM)

Solution II-Potassium per sulfate solution (17 mM)

0.3 ml of solution II was added to 50 ml of solution I. left at room temperature overnight in dark before use. 13.5 mg of the extracts and the standards were separately dissolved in 2 ml of DMSO and serially diluted to lower concentration. To 0.2 ml of various concentrations of the extract and standards, 1 ml of distilled DMSO and 0.16 ml of ABTS solution were added to make a final volume of 1.36 ml. Absorbance was measured after 20 min at 734 nm using ELISA reader against corresponding Blank solution. IC_{50} value obtained is the concentration of the sample required to inhibit 50% ABTS radical.

Lipid peroxidation (LPO) assay[2,5]

The test samples (100 µl) of different concentrations were added to 1 ml of egg lecithin mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl Ferric Chloride (400 mM) and 10 µl L-ascorbic acids (200 mM). After incubation for 1 hour at 37^0C, the reaction was stopped by the addition of 2 ml HCL(0.25 N) containing 15% TCA and 0.375% TBA and the reaction mixture was boiled for 15 min, cooled, centrifuged and the absorbance of the

TABLE NO:1

INVITRO ANTIOXIDANT ACTIVITY OF SELECTED ANTIASTHMATIC HERBAL CONSTITUENTS

S.NO	SAMPLES (Extracts & Phytoconstituents) & STANDARDS	IC_{50} values ±SE (µg/ml) by methods				
		DPPH	H_2O_2	ABTS	LPO	NO
1	Forskolin (95%)	> 1000	> 1000	285±0.36	> 1000	328±0.24
2	Piperine (98%)	>1000	>1000	436±0.33	>1000	246±0.17
3	Curcumin C_3 Complex (98.5%)	168±0.31	570.7±0.23	275±0.09	170±0.36	340±0.16
4	Curcumin C_3 complex + Piperine	936±0.34	879.16±0.25	486.5±0.11	171.9±0.32	325±0.09
5	Curcumin C_3 complex + Forskolin	826±0.19	973.5±0.21	245.6±0.33	170.2±0.36	265.5±0.36
6	Curcumin C_3 complex + Vasaka	914±0.36	868.4±0.09	322±0.13	170.3±0.19	412.5±0.33
7	Vasaka extract (75% Vasicinone)	>1000	>1000	467±0.33	>1000	382.5±0.12
8	Coleus extract (10% Forskolin)	>1000	>1000	30±0.26	>1000	235±0.32
9	Piper longum extract (20%Piperine)	>1000	>1000	215±0.08	>1000	30±0.27
10	STD-Ascorbic acid	56±0.35	172.36±0.14	35±0.22	—	30±0.27
11	STD-Rutin	53±0.28	46.15±0.16	125±0.28	—	185.5±0.09
12	α – tocopherol	—	—	—	65±0.36	—

Average of three determinations

supernatant was measured at 532 nm.

In all the above methods the IC_{50} value was calculated using the formula;

$$\% \text{ Inhibition} = \frac{\text{Absorbance (control)} \quad \text{Absorbance (sample)}}{\text{Absorbance (control)}} \text{X100}$$

RESULTS:

Among the selected phytoconstituents and extracts, Forskolin (95%), Piperine (98%) and Vasicinone (75%) showed a moderate antioxidant activity under ABTS and nitrous oxide method. The above three constituents exhibited better anti oxidant property with the combination of Curcumin C3 complex (98.5% Curcumin) in the ratio 1:1 under ABTS , Hydrogen peroxide , DPPH and Nitrous oxide method. IC50 values were comparable with that of the standard.

Coleus extract (10% Forskolin), Piper longum extract (20%Piperine) and Vasaka extract (30%Vasicinone) exhibited potent antioxidant activity among all the test samples showed a moderate antioxidant activity under ABTS and nitrous oxide method.

CONCLUSION:

This study suggests that the selected herbal based anti asthmatic constituents and extracts play a vital role in scavenging nitric oxide which could prevent the bronchial inflammation in asthmatic patients. Because nitric oxide is naturally formed in activated macrophages and endothelial cells and it is considered as an active agent in several pathologies based on inflammation. The selected herbal anti asthmatic phytoconstituents along with Curcumin C3 complex containing Curcumin, Desmethoxy Curcumn, and Bisdesmethoxy Curcumin showed potent antioxidant activity under DPPH, Lipid per oxidation, ABTS and Hydrogen peroxide method. Thus the study concludes stating that the selected phytoconstituents, either alone or in combination with curcuminoids can be utilized in alternate therapy in the treatment of bronchial asthma.

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