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Antioxidant and Antimicrobial Activity of Rhizome of Curcuma aromatica And Curcuma Zeodaria, Leaves of Abutilon Indicum.

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To compare the antimicrobial and antioxidant activity of the curcuma aromatica, curcuma Zeodaria, and Abutilon indicum leaves. Antimicrobial activity was carried out by Minimum inhibitory concentration method and antioxidant studies were carried out by DPPH, Nitric oxide method, and reducing power method. The hydro ethanolic extract of Curcuma Zeodaria and Curcuma Aromatica rhizomes were found to have potent antimicrobial activity against *Bacillus cereus* at 1000 µg/ml and showed moderate activity against *Klebselia pneumonia* and *Candida albicans*. All other extract did not show antimicrobial activity against any of the selected microorganism. In the case of MIC, hydro ethanolic extract of Curcuma aromatica inhibited *Bacillus cereus* at 15.625 µg/ml, *Klebselia pneumonia* was inhibited at 62.5 µg/ml and *Candida albicans* at125 µg/ml. Hydro ethanolic extract of curcuma Zeodaria inhibited *Bacillus cereus* at 31.25 µg/ml where as *Klebselia pneumonia* and *Candida albicans* were inhibited at 125 µg/ml. both hot and cold maceration product of hydro ethanolic extract of Curcuma aromatica rhizome showed potent antioxidant activity. All other extracts showed moderate to poor antioxidant activity. In the Nitric oxide method Curcuma aromatica rhizome extract showed moderate antioxidant activity for hot and cold hydroethanolic extract. All other extracts nearly failed to inhibit nitric oxide radical production. In reducing power method, among the nine extracts, hydro ethanolic extract prepared by hot maceration process of Curcuma aromatica showed highest antioxidant activity. All other extracts showed moderate to poor reducing activity. In total antioxidant capacity by phosphomolybdenum method, 50 % hydro ethanolic extract prepared by both hot and cold maceration process of Curcuma aromatica showed moderate antioxidant activity. All other extracts showed moderate to poor activity in mM equivalent of Ascorbic acid. While comparing the antioxidant activity, curcuma Zeodaria and curcuma aromatica found to possess potent antimicrobial activity, where as curcuma aromatica rhizomes found to possess potent antioxidant activity.

Key words: Antimicrobial, Antioxidant, Abutilon indicum, Curcuma aromatica, Curcuma Zeodaria.

INTRODUCTION

The free radical mediated damage may play in many disorders, in particular CHD, diabetes and cancer. Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structures and are therefore highly reactive. Oxygen is the most ubiquitous of all biologically important chemicals species and is a major source of reactive oxygen species (ROS). It has been estimated that up to 5 % of inhaled oxygen becomes an active oxygen species. Oxidative stress results from an imbalance between the oxidant production and antioxidant defenses. Increased oxidative stress is associated with many of the risk factors implicated in the pathophysiology of atherosclerosis, including diabetes, hypercholesterimia, renal failure, aging, hypertension and smoking. ROS include the superoxide anion (O_2), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH-), the peroxynitrite (OONO) radical. In its more severe form, the redox imbalance may result in cell death following wide spread macro molecule oxidation, while more subtle changes appear to play a role in modulating a range of signal transduction pathways. All the molecules are potential targets for ROS (Proteins, lipids and DNA) but because of their ubiquitous distribution within cell membranes and their propensity to contain double bond, unsaturated lipid are often targeted [1.] (Stephens JW et.al, 2009).

To protect the cells and organ systems of the body against reactive oxygen species, the human body has evolved a highly complex antioxidant protection system. It actually involves a variety of compounds, endogenous and exogenous, that

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Table 1. Antimicrobial activity of Curcuma Aromatic rhizomes by cup- plate method

Test Organisms	Zone of inhibition in mm										
	Curcuma aromatica rhizome										
	Hydro ethanolic extract prepared by hot maceration process (µg/ml)			Hydroethanolic extract prepared by cold maceration process (µg/ml)			Aqueous extract prepared by percolation process			Solvent (DMSO)	Standard drug
	1000	500	250	1000	500	250	1000	500	250		
Gram positive Bacteria											Tetracycline 25 µg/ml
Bacillus cereus	18	16	13	20	18	15	04	00	00	00	25
Staphylococcus aureus	05	03	00	06	04	00	00	00	00	00	35
Gram negative bacteria.											
Escherichia coli	00	00	00	00	00	00	00	00	00	00	20
Klebselia Pneumonia	06	04	00	07	05	00	00	00	00	00	19
Fungi											Amphotericin-B 15µg/ml
Candida albicans	03	00	00	04	00	00	00	00	00	00	11
Aspergillus niger	00	00	00	00	00	00	00	00	00	00	08

function interactively and synergically to neutralize free radicals.[2] (Zheng J et.al, 2008).Present scenario is showing the replacement of synthetic agents with herbals in nearly all filed of medicine. Antimicrobial and antifungal agents are also a part of this. The demand of natural food additives is increasing day and day. So herbs and species can be a better option for the replacement of synthetic antimicrobial agents. In general herbs containing essential oil, high flavonol and phenol content may possess strong antimicrobial properties[3.] (Nambisan B et.al 2005). This paper deals with the antimicrobial and antioxidant activity of the rhizome of curcuma aromatica and abutilon indicum leaves.

MATERIALS AND METHODS

The Rhizome of Curcuma aromatica, Curcuma Zeodaria, and leaves of Abutilon indicum were purchased from PSS Herbs, Pvt, Ltd, Kerala, India. The Medias which we used for the antimicrobial studies were purchased from Hi Media, and microorganism from Pune, and all other chemicals used were of analytical grade. Antimicrobial activity of the selected medicinal plants were performed by cup plate method and Minimum Inhibitory concentration method (Nambisan B et.al 2005)

In vitro antioxidant studies

A number of different methods may be necessary to adequately assess in vitro antioxidant activity of a specific compounds or antioxidant capacity of the biological fluids. Hence all the extracts were tested of in vitro antioxidant activities using several standard methods. In all these methods, the absorbance was measured against the corresponding blank solution and IC50 values were determined. IC_{50} is the concentration of the sample required to scavenge 50 % of free radicals was calculated.

Table 2. Antimicrobial activity of Curcuma Zeodaria rhizomes by cup- plate method

Test Organisms	Zone of inhibition in mm										
	Curcuma aromatica rhizome										
	Hydro ethanolic extract prepared by hot maceration process (µg/ml)			Hydroethanolic extract prepared by cold maceration process (µg/ml)			Aqueous extract prepared by percolation process			Solvent (DMSO)	Standard drug
	1000	500	250	1000	500	250	1000	500	250		
Gram positive Bacteria											Tetracycline 25 µg/ml
Bacillus cereus	21	19	16	22	20	18	00	00	00	00	25
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00	35
Gram negative bacteria.											
Escherichia coli	00	00	00	00	00	00	00	00	00	00	20
Klebselia Pneumonia	10	09	06	10	08	04	00	00	00	00	19
Fungi											Amphotericin-B 15µg/ml
Candida albicans	09	07	04	09	08	05	00	00	00	00	11
Aspergillus niger	00	00	00	00	00	00	00	00	00	00	08

Table 3. Antimicrobial activity of Abutilon indicum leaves by cup- plate method

Test Organisms	Zone of inhibition in mm										
	Curcuma aromatica rhizome										
	Hydro ethanolic extract prepared by hot maceration process (µg/ml)			Hydroethanolic extract prepared by cold maceration process (µg/ml)			Aqueous extract prepared by percolation process			Solvent (DMSO)	Standard drug
	1000	500	250	1000	500	250	1000	500	250		
Gram positive Bacteria											Tetracycline 25 µg/ml
Bacillus cereus	00	00	00	00	00	00	00	00	00	00	25
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00	35
Gram negative bacteria.											
Escherichia coli	00	00	00	00	00	00	00	00	00	00	20
Klebselia Pneumonia	00	00	00	00	00	00	00	00	00	00	19
Fungi											Amphotericin-B 15µg/ml
Candida albicans	00	00	00	00	00	00	00	00	00	00	11
Aspergillus niger	00	00	00	00	00	00	00	00	00	00	08

Table 4. Minimum Inhibitory Concentration of Curcuma Aromatica, Curcuma zeodaria rhizomes and Abutilon indicum leaves

Test Organisms	Minimum Inhibitory Concentration in µg/ml								
	Curcuma Aromatica rhizome			Curcuma Zeodaria rhizome			Abutilon indicum leaves		
	Hydro ethanolic extract prepared by hot maceration process	Hydro ethanolic extract prepared by cold maceration process	Aqueous extract prepared by percolation process	Hydro ethanolic extract prepared by hot maceration process	Hydro ethanolic extract prepared by cold maceration process	Aqueous extract prepared by percolation process	Hydro ethanolic extract prepared by hot maceration process	Hydro ethanolic extract prepared by cold maceration process	Aqueous extract prepared by percolation process
Gram positive Bacteria									
Bacillus cereus	31.25	15.625	>1000	31.25	31.25	>1000	>1000	>1000	>1000
Staphylococcus aureus	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Gram negative bacteria.									
Escherichia coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Klebselia Pneumonia	62.5	62.5	>1000	125	125	>1000	>1000	>1000	>1000
Fungi									
Candida albicans	125	125	>1000	125	125	>1000	>1000	>1000	>1000
Aspergillus niger	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

Table 5. In vitro antioxidant activity of Curcuma Aromatica, Curcuma Zeodaria rhizomes and Abutilon indicum leaves

Plant Extracts		DPPH method IC_{50} (µg/ml)	Nitric Oxide method IC_{50} (µg/ml)	Reducing power (µg/ml)	Total antioxidant capacity (mM equivalent of Ascorbic acid
Curcuma aromatica rhizome	50 % Hot EtOH	37.45 ± 2.5	372.27± 3.23	3.717±0.065	107.2±3.923
	50% cold EtOH	35.93± 2.105	339.16±2.03	2.332±0.003	87.6±2.02
	Aqueous extract	712.7±2.66	>1000	0.990±0.014	721.12±3.47
Curcuma Zeodaria Rhizome	50 % Hot EtOH	227.8±4.875	>1000	2.726±0.066	283 ±3.00
	50% cold EtOH	930±16.35	>1000	2.525±0.023	230.2±1.32
	Aqueous extract	757 ±13.5	>1000	0.575±0.034	872.52±5.62
Abutilon indicum Leaves	50 % Hot EtOH	156.95±7.07	728.63±2.03	2.289±0.058	231.4±3.69
	50% cold EtOH	219.2±3.89	823.79±2.95	2.298±0.061	258.6±2.71
	Aqueous extract	738.56±12.78	>1000	0.324±0.016	978.34±7.45
	Standards				
	Ascorbic acid	6.0±1.0	-	3.51 ± 0.0007	
	Rutin	11.75±0.48	88.47± 2.54		

The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control} - \text{OD Sample}}{\text{OD control}} \times 100.$$

Antioxidant activities of the selected medicinal plants were carried out by Diphenyl picryl Hydrazyl (DPPH) radical scavenging method. (Sanvarinda Y et.al, 2008) Nitric oxide radical inhibition activity [5] (Nanjan MJ et.al 2007) Reducing power assay [6, 7] (Arora S, et.al, 2007, Zhao M et.al 2007) Total antioxidant capacity [8] (Singab A.N.B et.al 2006)

RESULT AND DISCUSSIONS

Dried curcuma aromatica rhizome, curcuma Zeodaria and abutilon indicum leaves were powdered and macerated separately with 50 % ethanol by hot maceration, cold maceration and aqueous extract prepared by percolation. Qualitative phyto chemical analysis of curcuma aromatica rhizome showed the presence of the constituents including alkaloids, flavonoids and tannins in 50 % hydro ethanolic extract both in hot and cold where as alkaloids and flavonoids in aqueous extract, Curcuma Zeodaria rhizome showed the presence of the alkaloids in 50 % hydro ethanolic extract both in hot and cold. Abutilon indicum leaves showed the presence of the constituents including alkaloids, flavonoids, and steroids in 50 % hydro ethanolic extract both in hot and cold where as flavonoids were present in aqueous extract. 50 % hydro ethanolic extract prepared by hot and cold preparation of Curcuma aromatica showed to have high phenol content and flavonol content compared to Curcuma Zeodaria and Abutilon indicum leaves. 50 % hydro ethanolic extract prepared by both hot and cold maceration process of Curcuma aromatica, Curcuma Zeodaria and Abutilon indicum leaves were investigated for their antimicrobial activity against the gram positive bacteria such as *Bacillus cereus, Staphylococcus aureus* and gram negative bacteria such as *Escherichia coli, Klebselia pneumonia* and fungal strains such as *Candida albicans* and *Aspergillus niger*.Among all the extracts tested for Curcuma aromatica hydro ethanolic extract at 1000µg/ml showed good activity against *Bacillus cereus* but were nearly inactive against gram negative bacterial and fungal strains tested where as aqueous extract was found to be inactive against the entire microorganism tested. Hydroethanolic extract of Curcuma Zeodaria showed potent antibacterial activity against *Bacillus cereus* and moderate activity against *Klebsiella pneumonia* and *Candida albicans* but had poor activity against the other strains tested. The results are tabulated in the Table.2. None of the extract of Abutilon indicum showed antimicrobial activity against any of the microbial strain tested. The results are tabulated in the table.3. MIC for the extract was done using the serial dilution method. Hydro ethanolic extract of both the rhizome showed IC against Bacillus cereus in the range of 15.625 µ to 31.25 µg/ml. The results were tabulated in table.4.

In vitro antioxidant activity were carried out to evaluate the antioxidant potency of different Curcuma aromatica, Curcuma Zeodaria and Abutilon indicum leaves.Among the nine extracts tested by DPPH method, 50 % hydro ethanolic extract prepared by hot and cold maceration of Curcuma aromatica showed potent antioxidant activity with IC_{50} 37.45±2.5 µg/ml and 35.93 ± 2.105 µg/ml respectively and 50 % hydro ethanolic extract prepared by hot maceration process of Abutilon indicum leaves shown to have moderate antioxidant activity with IC_{50} value of 156.95± 7.07 µg/ml. The other extracts showed moderate to poor antioxidant activity with a wide range of 219 µg/ml to 738 µg/ml when compared to standard Ascorbic acid and rutin.Among the extracts tested for Nitric oxide radical scavenging activity, 50 % hydro ethanolic extract of Curcuma aromatica showed moderate antioxidant activity with the IC_{50} value of 372.27 ± 3.23 µg/ml and 339.16 ± 2.03 µg/ml respectively. The other extracts showed poor antioxidant activity in range of 728 µg/ml to

>1000 µg/ml when compared to standard rutin.Among all the extracts tested for the reducing power, the hydro ethanolic extract of curcuma aromatica was found to have highest reducing power (3.717 ±0.065 µg/ml) when compared to standard Ascorbic acid (3.51 ± 0.0007 µg/ml). The other hydro ethanolic extract showed moderate reducing power ranging from (2.289 ± 0.061 µg/ml). Aqueous extract had very poor reducing power. Among all the nine extracts tested for the estimation of total antioxidant capacity (TAC), the hydro ethanolic extract prepared by cold maceration process showed good antioxidant activity which has more prominent in case of Curcuma aromatica and hydroethanolic extract prepared by hot maceration process of Curcuma aromatica. All other extracts showed antioxidant capacity in the range of 230.2± 1.32 mM equivalent to Ascorbic acid to 978.34 ±7.45 mM equivalent of Ascorbic acid

CONCLUSION

Among the extracts tested the hydro ethanolic extract of Curcuma Zeodaria and Curcuma Aromatica rhizomes were found to have potent antimicrobial activity against selected strains of microorganism Only in DPPH method, hydro ethanolic extract showed the potent antioxidant activity but not in any other antioxidant method.

Based on the observations that has been found out the rhizomes of Curcuma aromatic and Curcuma Zeodaria and the leaves of Abutilon indicum do not have the expected anti microbial and antioxidant capacity. Because of that it may have a very less therapeutic value for various diseases because of its poor antioxidant activities.

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