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Biochemical studies on *Curcuma amada* extracts

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ABSTRACT

Naturally occurring antioxidants have considerable importance in medicine and in food processing. In this work, the antioxidant activity of dried rhizomes extract of the spice Curcuma amada (Mango ginger), a unique spice having morphological resemblance with ginger (Zingiber officinale) but imparts a raw mango (Mangifera indica) flavour is studied by the inhibition of auto oxidation of linoleic acid in aqueous alcohol system and by DPPH method along with antibacterial activity against selected organisms studied by disc diffusion method were reported.

Keywords: DPPH, antioxidants, Curcuminoids, mango ginger and *Curcuma amada*

INTRODUCTION

The plant and plant products have enriched human civilization since time immemorial. Over the centuries humans have relied on plants for basic needs such as food, clothing, and shelter and medicine. Besides functioning as the energy source for animals, they provide raw materials for many phytochemical based industries such as pharmaceutical, textile, perfumery, flavour and food industries. The Indian subcontinent is well known for its rich heritage of herbs and spices. With different climates in different parts of the country, India produces a variety of spices and herbs, many of which are native to the subcontinent, while others were imported from similar climates and have since been cultivated locally for centuries. The production and export of herbs and spices still makes an important contribution to the Indian economy[1]. In addition to the aroma and flavor qualities, many of the spices are known to exert a wide range of beneficial physiological effects which is reflected in their use in traditional medicines. Medicinal uses of plant extracts form a part of many pharmacopoeias of the world. Even in the modern age, exploring the different pharmacological action of medicinal plants and isolation of bioactive constituents present in them constitute a major research area all over the world. In this communication we report the antioxidant and bactericidal activity of dried rhizomes extract of spice *Curcuma amada* , unique spice having morphological resemblance with ginger (*Zingiber officinale*) but imparts a raw mango (*Mangifera indica*) flavour[2].

MATERIALS AND METHODS

Preparation of the extracts

The following general procedure is followed for extracting mango ginger rhizomes with organic solvents. 5 g of dry rhizome powder was stirred with 50 mL of organic solvent for approximately 3 hours using a magnetic stirrer. The mixtures were then filtered through Whatman No.1 filter paper. This process was repeated twice and the filtrates were combined together and then dried in a rotary evaporator. The weight of dry extract obtained was noted. These samples were used for the antioxidant and antimicrobial activity studies. Aqueous extract was prepared in the same manner from which water was removed using a Dean Stark apparatus (azeotrope with benzene) and then dried in a rotary evaporator. It is used for the antioxidant studies.

Antioxidant activity

The antioxidant assay is usually carried out on model systems such as methyl-linoleate or linoleic acid and there are several methods to study the extent of activity[3]. In the thiocyanate method, the ability of lipid hydro peroxide (formed during the auto oxidation of lipids such as linoleic acid) to oxidize $Fe^{2+} \rightarrow Fe^{3+}$ is exploited. In this method, the sample and linoleic acid in a water-ethanol medium is incubated at 40°C in the dark and the auto oxidation is followed at intervals by measuring the absorbance (at 500 nm) of the red colour developed after the addition of $FeCl_2$ and NH_4SCN. As the concentration of Fe^{3+} and hence absorbance depends on the extent of lipid autoxidation, the antioxidant activity can be judged qualitatively from a comparison of the absorbance of a control maintained under identical conditions[4]. The antioxidant assay procedure employed is given below.

A solution (2.53%) of linoleic acid in 99.5% ethanol and 0.05 M phosphate buffer of pH 7 were prepared. Solution (4mL) of the test compound (2 mg in 99.5% ethanol) was added to a solution mixture containing linoleic acid (4.1mL)., phosphate buffer (8mL) and distilled water (3.9 L) taken in stoppered Erlenmeyer flasks, and incubated at 40°C in the dark for 7-8 days. At periodic intervals during the incubation. 100 μL of the mixture was used for antioxidant assay by the thiocyanate method as described below.

The incubated solution (100 μL) was added to 75% ethanol (9.7ml) and 30% ammonium thiocyanate (0.1ml). Ferrous chloride solution (0.1mL, $2x10^{-2}$ M) in 3.5% HCl wasthen added and precisely after 3min, the absorbance of the red colour developed was measured at 500 nm. The antioxidant activity was judged from the decrease in the absorbance compared to the absorbance of a control maintained under identical condition.

DPPH is a well known radical to monitor chemical reactions involving radicals and recently it is most widely used for antioxidant assay. When a solution of DPPH having a strong absorption at 517 nm is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form of DPPH which can be monitored by measuring the absorbance at 517 nm. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The methanolic extract of mango ginger rhizome was tested for the scavenging effect on DPPH radical according to the method of Pan *et al*[5].

In this method, 0.2 mL of extract solution in ethanol (95 %) at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}) was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–VISIBLE spectrometer . All determinations were performed in triplicate. The DPPH radical scavenging activity (*S %*) was calculated using the following equation:

$$S\% = ((A_{\mathbf{control}} - A_{\mathbf{sample}})/A_{\mathbf{control}}) \times 100$$

where $A_{\mathbf{control}}$ is the absorbance of the blank control (containing all reagents except the extract solution) and $A_{\mathbf{sample}}$ is the absorbance of the test sample.

Antibacterial activity

Nutrient agar slants were used for maintaining the stock culture of test bacteria. A loopful of bacteria was transferred into 5 mL of nutrient broth (sterilized) and was incubated at 37^0 C for 6-8 hrs. From this culture, 200 µL of suspension was transferred to petriplates containing nutrient agar and spreaded evenly on the medium with the help of a glass spreader to get a uniform lawn of bacteria. Using an agar punch, wells were made on these seeded plates and 75µLs (4000ppm) of the test compounds as a solution in DMSO were added to the wells and each well were labeled. The petriplates were prepared in duplicate and inoculated at 37^0 C overnight. Ciprofloxin is used as the control in the solvent DMSO. The antimicrobial activity was determined by measuring the diameter of the zone inhibition (mm).

RESULTS AND DISCUSSION

The different solvents used for extraction and the mass of dried matter obtained from 5g of powdered rhizome are given in Table 1. 30 mg of dichloromethane extract of mango ginger extract was subjected to GC MS analysis. The chromatogram is given in Figure 1. Some of the peaks identified by comparison with literature available along with the Chemical library at Department of Chemistry, IIT Bombay and corresponding retention time are given in Table 2.

Analysis of the chemical composition of the extract by GC-MS facilitated the identification of components in dichloromethane extract (Table 2). The major compounds identified were curcumene, α-curcumene, ß-curcumene,

camphor, curzerenone , 1, 8-cineole ,Curcumin, demethoxy curcumin, bis-demethoxy curcumin, caffeic acid, ferulic acid ,gallic acid ,cinnamic acid , p-coumaric acid and gentisic acid.

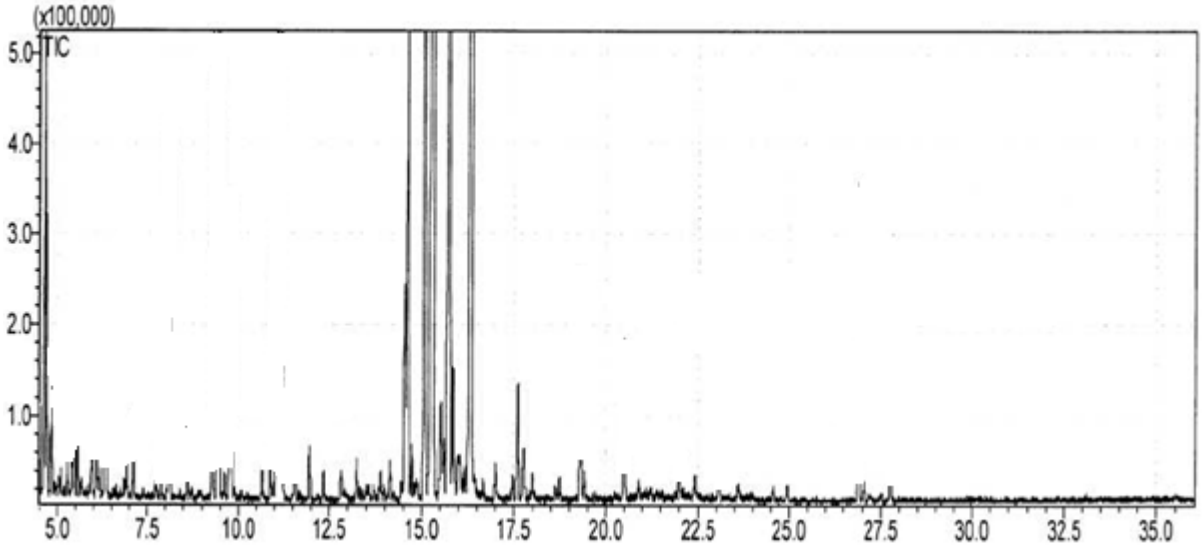

Figure 1 Chromatogram of dichloromethane extract of mango ginger

Table 1. The mass of crude extract using different solvents

Solvent	Mass of dry extract (in g)
Ethanol	0.50
Hexane	0.35
Ethyl acetate	0.30
Dichloromethane	0.25
Acetone	0.20
Water	0.20

Table 2: Phytochemicals identified from 100% dichloromethane extract of Mango Ginger rhizomes

S. No	Name	Relative Area%	RT	Molecular Formula	MW
1	α-curcumene	9.21	1.48	$C_{15}H_{22}$	202.17215
2	ß-curcumene	8.47	14.28	$C_{15}H_{24}$	204.1878
3	camphor	7.15	12.04	$C_{10}H_{16}O$	152.1201
4	curzerenone	8.18	14.08	$C_{15}H_{18}O_2$	230.1307
5	1, 8-cineole	6.45	13.37	$C_{10}H_{15}O$	154.1358
6	Curcumin	4.24	14.01	$C_{21}H_{20}O_6$	368.1260
7	demethoxy curcumin	1.85	15.45	$C_{20}H_{18}O_5$	338.1154
8	bis-demethoxy curcumin	1.28	16.21	$C_{19}H_{16}O_4$	308.1049
9	caffeic acid	9.25	15.45	$C_9H_8O_4$	180.0423
10	ferulic acid	8.28	15.58	$C_{10}H_{10}O_4$	194.0579
11	gallic acid	9.64	15.57	$C_7H_6O_5$	170.0215
12	cinnamic acid	9.14	15.27	$C_9H_8O_2$	148.0524
13	p-coumaric acid	7.22	12.45	$C_9H_8O_3$	164.073
14	unknown	5.49	17.21	$C_{14}H_{16}O_4$	248.1049
15	unknown	4.09	13.44	$C_{10}H_{12}O_3$	180.0786

Table 3 Absorbance data of BHT and mango ginger extracts

Number of days	BHT	Ethanol(1)	Hexane(2)	Ethyl ace
1	0.02	0.02	0.03	0.02
2	0.04	0.08	0.06	0.04
3	0.06	0.19	0.11	0.07
4	0.11	0.37	0.18	0.11
5	0.16	0.51	0.27	0.21
6	0.23	0.72	0.39	0.31
7	0.31	0.95	0.49	0.48

Antioxidant activity

All the extracts prepared were tested for their antioxidant activity by ferric thiocyanate method. However, only dichloromethane extract was selected for studying the DPPH scavenging activity. The absorbance data in ferric

thiocyanate method is given in Tables 3-4. It is graphically represented in Figures 2-3. The results show that the ethyl acetate and dichloromethane extracts have maximum antioxidant activity probably due the solubility of phenolic compounds. The absorbance data in DPPH method is given in Table 5 and graphically represented in Figure 4.

Table 4 Absorbance data of BHT and mango ginger extracts

Number of days	BHT	Dichloromethane(4)	Acetone(5)	Water(6)
1	0.02	0.02	0.02	0.02
2	0.04	0.04	0.04	0.05
3	0.06	0.07	0.09	0.11
4	0.11	0.11	0.17	0.25
5	0.16	0.19	0.25	0.39
6	0.23	0.31	0.39	0.52
7	0.31	0.48	0.52	0.72

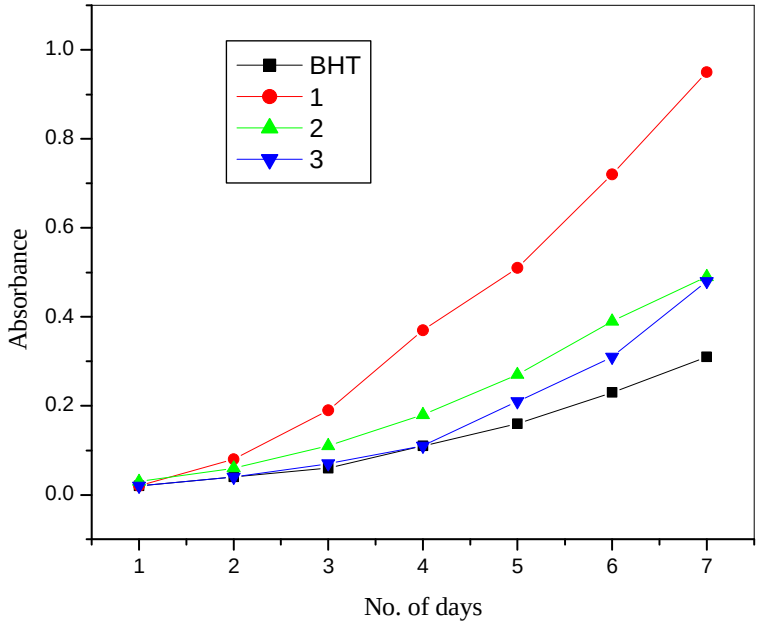

Figure 2. Absorbance data of BHT and mango ginger extracts (1.Ethanol 2.Hexane and 3.Ethyl acetate)

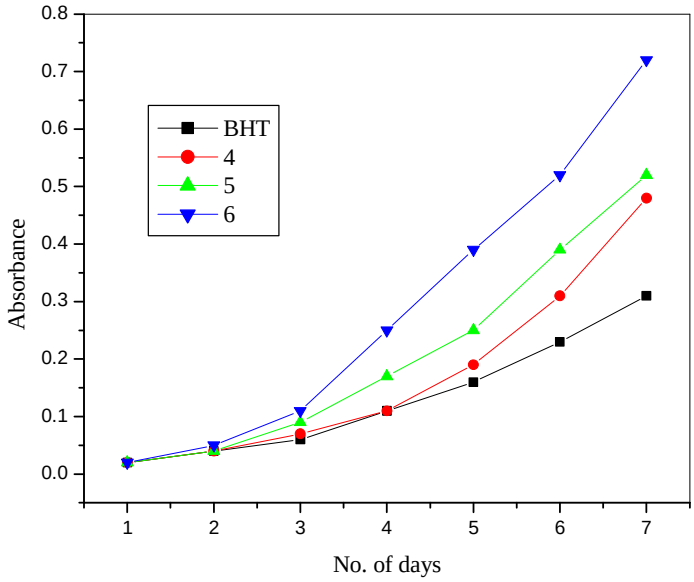

Figure 3 Absorbance data of BHT and mango ginger extracts (4.dichloromethane, 5. Acetone and 3.Water)

Table 5.DPPH scavenging activity

Sl. No.	Concentrations (mg mL^{-1})	Absorbance of control	Absorbance of sample	S%
1	0.2	0.74	0.65	12.162
2	0.4	0.74	0.54	27.027
3	0.6	0.74	0.42	43.243
4	0.8	0.74	0.31	58.108

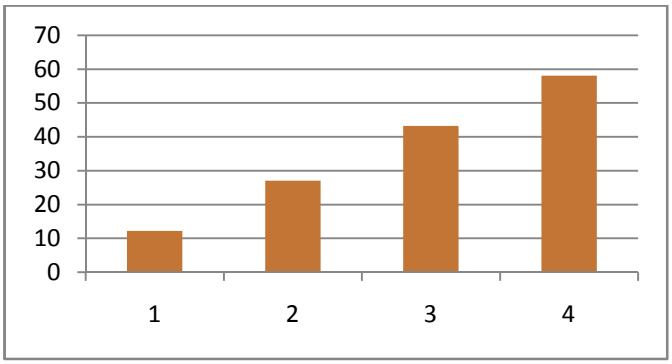

Figure 4 DPPH scavenging activity (%S against concentration)

Antibacterial assay

The organism selected for studying the antibacterial activity are *Staphylococcus aureus* (ATCC 25923), *Pseudomonaes aeruginosa* (ATCC 27853) and *Escherischchia coli* (ATCC 25922). The Staphylococcus constitute and ubiquitous group of microorganism that may be detected in air, dust and natural water. One species, *S.aureua* (a gram positive organism) is particularly associated with human beings and found on the skin and mucous membranes of nose and throat. The organism wills apart to grow in food material and secrete enterotoxins which show considerable resistance to the proteolytic digestive enzymes. The victim suffers from vomiting and sometimes diarrhea accompanied by sweating, fever, hypothermia, headache and muscular cramps. E. coli is a gram negative aerobic bacterium usually present in the lower portion of the intestine of warm blooded animals including human beings. Certain E. coli strains can cause gastroenteritis, urinary tract infections and pyogenic infections[6].The diameter of the zone inhibition (mm) are given in Table 6. From the results it follows that hexane, dichloromethane and ethyl acetate extracts were more effective against the tested organisms.

Table 6 Average diameter of the zone of inhibition

Extracts	Average diameter(mm) of the zone of inhibition		
	S.aureus	*P. aeruginosa*	*E.coli*
Hexane	13	10	12
Ethyl acetate	11	10	10
Dichloromethane	12	11	13
Acetone	10	9	7
control	32	31	32

CONCLUSION

The major components present in mango ginger rhizome extracted with dichloromethane were identified by GC MS. The potential antioxidant activity of mango ginger extracts were established by ferric thiocyanate method and DPPH scavenging method. Our studies reveal that ethyl acetate and dichloromethane extracts have the maximum antioxidant activity. Among the extracts screened for antimicrobial activity, hexane, dichloromethane and ethyl acetate extracts showed maximum activity against all tested organism. Owing to the unique nature of mango ginger and its wide pharmacological activities, further studies are required to establish the actual bioactive component in the extract.

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