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Analysis of Phytochemical Constituents, Pharmacological and Ethnobotanical Studies of Selected Folk Medicinal Plant, *Curcuma amada* Roxb. (Mango Zinger)

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Abstract

Curcuma amada, also known as Mango ginger, is a distinctive spice that shares morphological similarities with ginger but imparts a raw mango flavor. The primary use of *Curcuma amada* rhizome lies in pickle and culinary preparations. In traditional medicinal systems such as Ayurveda and Unani, *Curcuma amada* is highly valued for its diverse properties, serving as an appetizer, alexteric, antipyretic, aphrodisiac, diuretic, emollient, expectorant, and laxative. It is prescribed to address various conditions, including biliousness, itching, skin diseases, bronchitis, asthma, hiccough, and inflammation from injuries. Despite its extensive traditional use, there is a scarcity of studies exploring the antioxidant, antimicrobial, and cytotoxic activities of *Curcuma amada* rhizome extract. Antibacterial activity was assessed using the agar well diffusion method against Gram-positive *Staphylococcus aureus* and three Gram-negative strains- *Klebsiella pneumonia, Escherichia coli, and Acetobacter aceti*. The study aims to investigate the antimicrobial and cytotoxicity activities, as well as conduct in silico pharmacological analysis of *Curcuma amada*. In antibacterial tests, a notable zone of inhibition was observed against *Acetobacter aceti and Escherichia coli*, while none was observed against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Cytotoxicity was assessed through a brine shrimp lethality bioassay, revealing results based on mortality numbers and the percentage of affected brine shrimp. In the in silico analysis, which evaluates pharmacological activity through pharmacokinetic analysis and molecular docking, four phytochemicals (E)-.beta.-Farnesene, eupatin, 3-Carene, and coumarin from the plant were selected. These phytochemicals were docked against the Estrogen receptor alpha (ERα), a protein associated with breast cancer. The docking results demonstrated promising binding scores, with E-.beta.-Farnesene, eupatin, 3-Carene, and coumarin scoring -11.3, -6, -6.3, and -8.1, respectively.

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Keywords: *Curcuma amada*, antimicrobial activity, Cytotoxicity assay, Disc diffusion method, *in silico* study.

Introduction

Since ancient times, medicinal plants have served as a rich reservoir of antimicrobial agents (Mahesh and Satish, 2008). In rural areas, traditional medicine derived from plant materials is widely utilized due to its easy accessibility and cost-effectiveness compared to modern medicine (Rashedul et al., 2010). Plants synthesize diverse secondary metabolites, including phenols, alkaloids, terpenoids, and glycosides, which play pivotal roles as sources of microbicides, pesticides, fungicides, and pharmaceutical drugs (Srinivasan et

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al., 2001). Traditional medicine predominantly relies on plant products as the primary source of pharmaceutical agents (Srinivasan et al., 2001).

The Zingiberaceae family, renowned for its wealth of phytochemical substances, is particularly abundant in curcuminoids with diverse chemical structures and varied physicochemical characteristics (Revathy et al., 2011). These plants, widely used in traditional medicine, exhibit a broad range of biological activities (Anand 2008). Numerous Curcuma species, including *Curcuma longa, Curcuma zedoaria, Curcuma aromatic, and Curcuma amada*, have been reported in literature for their antifungal, antibacterial, and anti-inflammatory properties (Apisariyakul et al., 1995; Yoshioka et al., 1998). The rhizomes of these plants, used for centuries in traditional medicine, are valued for their medicinal effects (Wibowo et al., 2012).

Curcuma amada, commonly known as mango ginger, belongs to the Zingiberaceae family and is closely related to turmeric (Curcuma longa). Its rhizomes share similarities with common ginger but lack the pungent taste, offering a distinct raw mango flavor. Coined by Linnaeus in 1753, the genus name Curcuma likely originates from the Arabic word 'kurkum,' meaning yellow color (Salvi et al., 2000; Shirgurkar et al., 2001). As an aromatic, perennial rhizomatous plant, Curcuma amada is part of a larger family comprising 70–80 species of rhizomatous annual or perennial plants (Purseglove 1974; Aminul 2004). The genus is extensively dispersed throughout the tropical regions of Asia, Africa, and Australia, having its origins in the Indo-Malayan area (Sasikumar 2005). In north India, they are used to make chutneys, and in south India, pickles. Plant-derived extracts have long been seen as a valuable medicine for preventing and treating chronic illnesses, as well as for preserving general immunological function (Alzoreky, N. *et al.*, 2003). In food processing, extracts from spices, fragrant herbs, and medicinal plants are used to provide taste and other useful qualities.

Figure 1. Collected rhizome of *Curcuma amada*

Phytochemicals that are found naturally in fruits, vegetables, and spices have the ability to either prevent or lower the risk of several illnesses (Bartz, J. A. *et al.*, 2002). The scientific confirmation of the widespread usage of polyphenols, terpenoids, alkaloids, flavonoids, and other secondary metabolites in plants will be achieved through their existence (Nasar-Abbas, S. *et al.*, 2004). The Curcuma genus encompasses more than 80 rhizomatous herb species, with origins in the Indo-Malayan region and widespread distribution across the tropical regions of Asia, Africa, and Australia (Policegoudra, R. et al., 2008). *Curcuma amada*, with a distinctive appearance similar to ginger, is commonly utilized for pickling, adding a raw mango flavor to cuisine, and for medicinal purposes (Sasikumar 2005). In the ancient Indian system of medicine, Ayurveda, the rhizome is valued for its properties as an appetizer, alexiteric, antipyretic, aphrodisiac, and laxative (Shankaracharya 1982). It finds application in the treatment of various conditions such as biliousness, itching, skin diseases, bronchitis, asthma, hiccups, and injuries-induced inflammation. The Unani system attributes diuretic, maturant, emollient, expectorant, antipyretic, and appetizer qualities to *Curcuma amada*, recommending it for ailments like inflammation in the mouth and ear, gleet, ulcers on male sex organs, scabies, lumbago, and stomatitis (Warrier 1994).

Worldwide, infectious diseases pose significant health risks (Madhuri, S. et al., 2009). *Staphylococcus aureus* is a prevalent microorganism causing skin diseases, while *Escherichia coli* is a well-known member of the human intestinal microbiota and a versatile

gastrointestinal pathogen. Zingiberaceae family plants, including *Curcuma amada*, are renowned for their preservative and medicinal properties, suggesting the potential presence of antimicrobial compounds. A study on mango ginger's antibacterial properties, using both aqueous and organic solvent extracts, demonstrated efficacy against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* (Chandarana et al., 2005). Notably, the heated aqueous extract exhibited higher antibacterial activity compared to the unheated counterpart. Solvent extracts, particularly 1, 4-dioxan and DMF, showed lower efficacy against bacteria. Besides antibacterial effects, phenolic fractions of mango ginger effectively inhibited H+, K+-ATPase activity and the growth of *Helicobacter pylori*. Specifically, cinnamic and ferulic acids in *Curcuma amada* were identified as significant contributors to these inhibitory effects. Various extracts, including hexane, chloroform, ethyl acetate, acetone, and methanol, displayed high antibacterial activity against several bacteria (Policegoudra et al., 2007a, b). Recently, difurocumenonol and amadannulen, two bioactive molecules, were isolated from mango ginger, exhibiting diverse bioactivities such as antibacterial activity, platelet aggregation inhibitory activity, cytotoxicity, and antioxidant activities like DPPH radical scavenging, lipid peroxidation inhibition, metal chelation, and superoxide radical scavenging (Policegoudra et al., 2007a, b).

Materials and Methods

Antibacterial activity of Curcuma amada rhizome extract

Sample collection

The plants rhizome was collected from different area at Kushtia District in Bangladesh. In our study, we work on mango ginger which smells like a raw mango. We have collected this mango ginger from Sheikhpara, Tribeni, Modhupur, Bittipara located area.

Sample preparation

The rhizome of *Curcuma amada* were washed thoroughly with double distilled water properly to remove dust, sands and other unnecessary particles and slice it properly by knife to dry almost 7-10 days at room temperature. After drying the samples, materials are blended by mechanical grinder to convert powder form. Then 5 g powder was measured using weight balance and takes it a conical flask. Then 25 ml Ethyl acetate are taken in sample to form solution and shake this solution by shaker for proper mixing this solution. Then, filter this sample to require the extract of this sample. Solvent extraction was carried out for 48 h in total. After each solvent extraction step, the extracts were filtered and concentrated by using rotary evaporator. The concentrated extracts were freeze-dried to remove the solvent and stored in refrigerator.

Figure 2. *Curcuma amada* dried rhizome and powder

Collection of organisms

Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Acetobacter aceti strains were collected from microbiology laboratory of Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

Media Preparation

In this study, we used 100 ml of Tryptone Soy Agar (TSA) media for inoculation in antimicrobial activity tests. The preparation involved suspending 4.5 grams of TSA in 100 ml distilled water, boiling to dissolve the medium, autoclaving for sterilization, cooling to 45-50°C, and then pouring into sterile Petri plates.

TSB broth preparation for Bacterial growth

We prepared 100 ml TSB broth in each conical flask, and I used four conical flask for four types of microorganism's growth. Weight 3 gm of Tryptic Soy Broth (TSB) powder and dissolve it 100 ml

distilled water Then mix it properly by glass rod and autoclave it 121º C for 45 min.

Disc diffusion method

Microbial strains underwent assessment for their sensitivity to antimicrobials utilizing the disc diffusion technique, as detailed by Bauer et al. in 1966. This method served to appraise the in vitro antibacterial and antifungal efficacy of the test sample against specific human pathogenic microorganisms. Tryptone Soy Agar (TSA) and other agar media were employed for the antibacterial and antifungal evaluations, respectively. The test solutions, derived from 5 g of Curcuma amada powder and 25 ml of ethyl acetate, were meticulously dispensed in varying volumes (50, 100, 150, and 200 µl) onto individual 6 mm diameter discs.

To initiate the procedure, 1000 µl of broth culture was evenly distributed to cover the entire surface area of the media. Subsequently, the plates were incubated at a temperature of 37±1°C for a duration of 24 to 48 hours. Following the incubation period, the resulting zones of inhibition were measured in terms of diameter (mm).

Screening Cytotoxicity of Curcuma amada

Collection of Plant Materials

The fresh *Curcuma amada* plant rhizome was collected from different area at Kushtia District in Bangladesh (Fig 1).

Preparation of Solvent Extracts

The rhizomes of *Curcuma amada* underwent a meticulous cleaning process, involving repeated washing with running water and a final rinse with sterile water. Subsequently, they were finely chopped, subjected to shade drying, and mechanically ground into a coarse powder using a grinder (refer to Fig 2). The resulting powder was sieved, and 50 grams of the dried material were subjected to extraction using ethyl acetate in a Soxhlet apparatus. The obtained extract underwent filtration through Whatman No. 1 filter paper, followed by concentration using a rotary flash evaporator. The concentrated extract was then stored in airtight bottles at 5º C for future use. These extracts were later evaluated for both antimicrobial and cytotoxic activities.

Cytotoxicity Test

The brine shrimp lethality assay was carried out based on the methodology outlined by Meyer et al. (1982), with minor adjustments. Brine shrimp eggs underwent hatching in 1% NaCl saline water, maintaining a pH of 8.5 and ensuring continuous aeration for a duration of 36 hours. Following hatching, 10 active nauplii were introduced into individual test tubes containing 20 gm/L saline water. Various concentrations of the rhizome extract (0, 25, 50, 100, and 200 ppm) were introduced into the test tubes, which were subsequently incubated for 24 hours under optimal conditions. The calculation of the LC50 (50% lethal concentration) involved a comparison between the mortality rates of nauplii in the experimental groups and the control group.

In Silico Study of Curcuma amada

Chemical Profiling

Information on the compounds of *C. amada* was gathered from various sources, including literature and web resources, to create an exclusive curated chemical library. Literature mining was conducted using international databases such as Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com/), Web of Science (https://www.webofscience.com/wos/woscc/basic-search), and PubMed (https://pubmed.ncbi.nlm.nih. gov/). The acquired data underwent a dual-check process for accuracy and was cross-referenced with available chemical databases like 'PubChem (https://pubchem.ncbi.nlm.nih.gov/)' and 'ChemSpider (http://www.chemspider.com/)' (Rösler et al., 2012). Details such as chemical name, IUPAC name, plant part, and structural information were systematically collected for the identified compounds.

Table 1. Antimicrobial activity of *Curcuma amada* at varied concentrations.

Name of Microorganisms	Dose concentration (µl/disc)	Zone of Inhibition (mm)
Acetobacter aceti	50	No clear zone
	100	3.2
	150	7.0
	200	8.2
Staphylococcus aureus	50	No clear zone
	100	No clear zone
	150	2.5
	200	3.5
Klebsiella pneumoniae	50	No clear zone
	100	No clear zone
	150	No clear zone
	200	3.8
Escherichia coli	50	No clear zone
	100	No clear zone
	150	5.4
	200	3.2

Determination of ADMET Properties

The computation of ADMET properties was carried out using Swiss ADME, accessible at http://www.swissadme.ch/. This online platform facilitates the calculation of physicochemical descriptors and prediction of ADME parameters, pharmacokinetic properties, drug-like characteristics, and medicinal chemistry compatibility for one or multiple small molecules. A set of 135 molecular descriptors was determined using SMILE numbers and chosen in accordance with Lipinski's rule of five. Lipinski's rule of five, also recognized as Pfizer's rule of five or simply RO5, is a heuristic guideline employed to assess drug likeness. It helps determine whether a chemical compound, exhibiting certain pharmacological or biological activity, possesses chemical and physical properties conducive to being an orally active drug in humans. Christopher A. Lipinski formulated this rule in 1997, noting the commonality that orally administered drugs are typically small and moderately lipophilic molecules (Lin et al., 2014).

Target Protein and Compounds Selection

Extensive literature exploration was conducted across global databases such as Google Scholar (https://scholar.google.com/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/). This comprehensive search aimed to identify compounds with promising potential for addressing breast cancer. The protein Estrogen receptor alpha (ERα) is responsible for breast cancer, so we selected this ERα for our target protein (Chou et al., 2020).

Table 2. Toxicity value of *C. amada* extract by *Artemia Salina*

Concentration (ppm)	No. of Brine shrimp	Mortality number (After 24hrs.)	Percentage (%) of Mortality (after 24 hrs.)
Control	10	0	0%
25	10	0	0%
50	10	0	0%
100	10	1	10%
200	10	2	20%

Molecular Docking

We used the CB-Docking a blind docking web server for finding out the interaction between our compound and receptors (https://www.nature.com/articles/s41401-019- 0228-6.pdf)., for *C. amada* we selected the ERα receptor for breast cancer. The CB-Dock web server employs an innovative

curvature-based cavity detection technique to predict binding sites for a given protein, calculating their centers and sizes. Subsequently, it conducts docking using Autodock Vina, a widely-used docking program. Through meticulous optimization, this approach demonstrates an impressive ~70% success rate for top-ranking poses, exhibiting a root mean square deviation (RMSD) within 2 Å from the X-ray pose. Notably, it outperforms existing blind docking tools in our benchmark tests. CB-Dock also provides an interactive 3D visualization of results. The determination of the best-docked complex with an appropriate pose is based on the energy score.

Results

Antimicrobial activity of Curcuma amada against some selected bacterial strains

This experiment was conducted for observing the zone of inhibition against *Acetobacter aceti* and *Escherichia coli* which are Gram negative bacteria, but zone of inhibition is not observed against *Staphylococcus aureus* and *Klebsiella pneumoniae* which one is Gram positive and another is Gram negative bacteria (Fig 3.1).

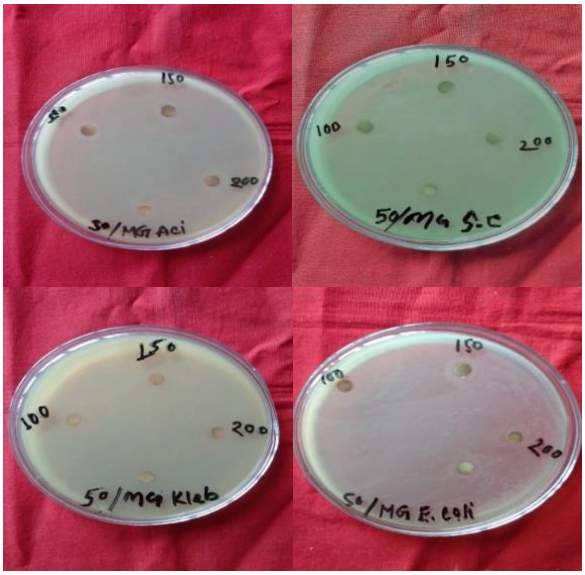

Figure 3.1. Antimicrobial activity of *Curcuma amada* extract against microorganisms (*A. aceti, S. aurues, K. pneumoniae, & E. coli*)

Results of Cytotoxicity Activity

The toxicity assessment level of *Curcuma amada* was measured by counting dead larvae of brine shrimp after 24 h of incubation. To assessment the toxicity level of this leaf extract, different concentration of leaf extracts was used such as- 25 ppm , 50 ppm, 100 ppm , 200 ppm. Through the calculation of LC_{50}, the concentration, 100ppm and 200ppm showed most toxicity value.

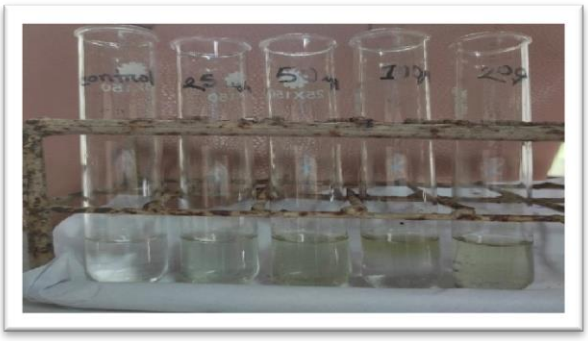

Figure 3.2. Toxicity assessment of Curcuma amada rhizome extract by *Artemia Salina*

Data Analysis

Depending on the experimental conditions, survivors were quantified either through the use of a magnifying glass or a microscope within a specified time frame of 24 hours. The determination of the median lethal concentration (LC50) for the test samples involved plotting the percentage of shrimp killed against the logarithm of the sample concentration, following the methodology outlined by Meyer et al. in 1982. Estimation of LC50 values employed probit regression analysis, as described by Finney et al. in 1971. Finney's statistical approach has been integrated into various software packages, including Stata, MATLAB, R, and IBM SPSS, facilitating automated LC50 calculations along with confidence intervals (refer to Table 2). While these computations may not yield the precise lethal concentration for a particular compound or extract causing 50% population mortality, they undoubtedly provide significant preliminary data for subsequent toxicity testing assays. Notably, most studies conducted the experiment in triplicate to ensure statistically reproducible results, as highlighted by Hamidi et al. in 2014.

Table 3. Phytochemical's profiling features of *C.amada*

No.	Phytochemical Name	Canonical SMILES	Drug likeness
1	(E)-Decahydronaphthalene	C1CCC2CCCCC2C1	Yes
2	(E)-Labda-8(17),13-diene-15,16-olide	CC1(CCCC2(C1CCC(=C)C2CCC3=CC(=O)OC3)C)C	Yes
3	(E)-Sabinol	CC(C)C12CC1C(=C)C(C2)O	Yes
4	(E)-Thujone	CC1C2CC2(CC1=O)C(C)C	Yes
5	(E,E)-alpha-farnesene	CC(=CCCC(=CCC=C(C)C=C)C)C	Yes
6	(Ethoxymethyl)benzene	CCOCC1=CC=CC=C1	Yes
7	(Z)-beta-Farnesene	CC(=CCCC(=CCCC(=C)C=C)C)C	Yes
8	(Z)-Farnesene	CC=C(C)C=CC=C(C)CCC=C(C)C	Yes
9	(Z,Z)-Alloocimene	CC=C(C)C=CC=C(C)C	Yes
10	1,8-Cineole	CC1(C2CCC(O1)(CC2)C)C	Yes
11	2,5-Dihydroxybenzoic acid	C1=CC(=C(C=C1O)C(=O)O)O	Yes
12	2,6-Dimethylhept-5-en-1-al	CC(CCC=C(C)C)C=O	Yes
13	2-Hydroxy-2'-Methoxy diphenyl ether / 2-(2-methoxyphenoxy)phenol	COC1=CC=CC=C1OC2=CC=CC=C2O	Yes
14	2-Methyl-6-methylene-3,7-octadien-2-ol	CC(C)(C=CCC(=C)C=C)O	Yes
15	2-Methyl-6-methyleneocta-1,7-dien-3-one / 2,6-Dimethyleneoct-7-en-3-one	CC(=C)C(=O)CCC(=C)C=C	Yes
16	2-Methylheptan-3-ol	CCCCC(C(C)C)O	Yes
17	2-Undecanone	CCCCCCCCCCC(=O)C	Yes
18	4-Terpineol	CC1=CCC(CC1)(C(C)C)O	Yes
19	6-Methyl-5-hepten-2-one	CC(=CCCC(=O)C)C	Yes
20	Acetone	CC(=O)C	Yes
21	alpha-Bergamotene	CC1=CCC2CC1C2(C)CCC=C(C)C	Yes
22	alpha-Copaene	CC1=CCC2C3C1C2(CCC3C(C)C)C	Yes
23	alpha-Fenchol	CC1(C2CCC(C2)(C1O)C)C	Yes
24	alpha-Guaiene	CC1CCC(CC2=C1CCC2C)C(=C)C	Yes
25	alpha-Humulene	CC1=CCC(C=CCC(=CCC1)C)(C)C	Yes
26	alpha-Ionone	CC1=CCCC(C1C=CC(=O)C)(C)C	Yes
27	alpha-Longipinene	CC1=CCC2C3C1C2(CCCC3(C)C)C	Yes
28	alpha-Muurolene	CC1=CC2C(CC1)C(=CCC2C(C)C)C	Yes
29	alpha-Muurolol	CC1CCC2C(C1)C(CCC2(C)O)C(C)C	Yes
30	alpha-Phellandrene	CC1=CCC(C=C1)C(C)C	Yes
31	alpha-Pinene	CC1=CCC2CC1C2(C)C	Yes
32	alpha-Selinene	CC1=CCCC2(C1CC(CC2)C(=C)C)C	Yes
33	alpha-Terpinene	CC1=CC=C(CC1)C(C)C	Yes
34	alpha-Terpineol	CC1=CCC(CC1)C(C)(C)O	Yes
35	Amadannulen	CCOC(=O)C1CC(CC(C1)C2CCCCC(CC3=C(C2)CC(C3)O)C)C	Yes
36	ar-Curcumene	CC1=CC=C(C=C1)C(C)CCC=C(C)C	Yes
37	ar-Turmerone	CC1=CC=C(C=C1)C(C)CC(=O)C=C(C)C	Yes

Continued

No.	Phytochemical Name	Canonical SMILES	Drug likeness
38	beta-Bisabolol	CC1=CCC(CC1)(C(C)CCC=C(C)C)O	Yes
39	beta-Caryophyllene	CC1=CCCC(=C)C2CC(C2CC1)(C)C	Yes
40	beta-Copaene	CC(C)C1CCC2(C3C1C2C(=C)CC3)C	Yes
41	beta-Curcumene	CC1=CCC(=CC1)C(C)CCC=C(C)C	Yes
42	beta-Elemene	CC(=C)C1CCC(C(C1)C(=C)C)(C)C=C	Yes
43	beta-Farnesene	CC(=CCCC(=CCCC(=C)C=C)C)C	Yes
44	beta-Gurjunene	CC1CCC2C(C2(C)C)C3C1CCC3=C	Yes
45	beta-Phellandrene	CC(C)C1CCC(=C)C=C1	Yes
46	beta-Pinene	CC1(C2CCC(=C)C1C2)C	Yes
47	beta-Selinene	CC(=C)C1CCC2(CCCC(=C)C2C1)C	Yes
48	beta-sitosterol	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	Yes
49	beta-Terpineol	CC(=C)C1CCC(CC1)(C)O	Yes
50	BisdemethoxyCurcumin	C1=CC(=CC=C1C=CC(=O)CC(=O)C=CC2=CC=C(C=C2)O)O	Yes
51	Borneol	CC1(C2CCC1(C(C2)O)C)C	Yes
52	Bornylformate	CC1(C2CCC1(C(C2)OC=O)C)C	Yes
53	Caffeic acid	C1=CC(=C(C=C1C=CC(=O)O)O)O	Yes
54	Camphene	CC1(C2CCC(C2)C1=C)C	Yes
55	Camphor	CC1(C2CCC1(C(=O)C2)C)C	Yes
56	Car-3-ene	CC1=CCC2C(C1)C2(C)C	Yes
57	Carvomenthene	CC1=CCC(CC1)C(C)C	Yes
58	Caryophyllene oxide	CC1(CC2C1CCC3(C(O3)CCC2=C)C)C	Yes
59	Cinnamic Acid	C1=CC=C(C=C1)C=CC(=O)O	Yes
60	cis-beta-Ocimene	CC(=CCC=C(C)C=C)C	Yes
61	cis-Dihydroocimene	CC(C)CCC=C(C)C=C	Yes
62	cis-Farnesal	CC(=CCCC(=CCCC(=CC=O)C)C)C	Yes
63	Citronellal	CC(CCC=C(C)C)CC=O	Yes
64	Coronarin B	CC1(CCCC2(C1CCC(=C)C2C3C=C(CC(OO3)O)C=O)C)C	Yes
65	Coronarin D	CC1(CCCC2(C1CCC(=C)C2CC=C3CC(OC3=O)O)C)C	Yes
66	Cubebol	CC1CCC(C2C13C2C(CC3)(C)O)C(C)C	Yes
67	Curcumin	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	Yes
68	Curzerene	CC1=COC2=C1CC(C(C2)(C)C=C)C(=C)C	Yes
69	Curzerenone	CC1=COC2=C1C(=O)C(C(C2)(C)C=C)C(=C)C	Yes
70	delta-3-Carene	CC1=CCC2C(C1)C2(C)C	Yes
71	delta-Elemene	CC(C)C1=CC(C(CC1)(C)C=C)C(=C)C	Yes
72	DemethoxyCurcumin	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC=C(C=C2)O)O	Yes
73	Difurocumenonol	CC1CC2=C(C(=CO2)C)C(=O)C3C(C(C=C1)O)(C4(C(C3(C5=C4C(C6=C(C5O)C(=CO6)C)C)C)O)O)C	Yes
74	Dihydromyrcenol	CC(CCCC(C)(C)O)C=C	Yes
75	Epicurzerenone	COC1=C(C=CC(=C1)C=CC(=O)O)O	Yes
76	Ferulic acid	COC1=C(C=CC(=C1)C=CC(=O)O)O	Yes
77	Gallic-acid	C1=C(C=C(C(=C1O)O)O)C(=O)O	Yes

Continued

No.	Phytochemical Name	Canonical SMILES	Drug likeness
78	gamma-Bisabolene	CC1=CCC(=C(C)CCC=C(C)C)CC1	Yes
79	gamma-Elemene	CC(=C1CCC(C(C1)C(=C)C)(C)C=C)C	Yes
80	gamma-Guaiene	CC1CCC(=CC2=C1CCC2C)C(C)C	Yes
81	gamma-Terpinene	CC1=CCC(=CC1)C(C)C	Yes
82	Geranial	CC(=CCCC(=CC=O)C)C	Yes
83	Geraniol	CC(=CCCC(=CCO)C)C	Yes
84	Geranylformate	CC(=CCCC(=CCOC=O)C)C	Yes
85	Germacrene D	CC1=CCCC(=C)C=CC(CC1)C(C)C	Yes
86	Germacrone	CC1=CCC(=C(C)C)C(=O)CC(=CCC1)C	Yes
87	Guaia-6,9-diene	CC1CCC2C1C=C(CC=C2C)C(C)C	Yes
88	Hept-1-en-1-ol acetate	CCCCCC=COC(=O)C	Yes
89	Isoborneol	CC1(C2CCC1(C(C2)O)C)C	Yes
90	Labdadiene	CC1(CCCC2(C1CCC(=C)C2CC=C(CC=O)C=O)C)C	Yes
91	Lavandulol	CC(=CCC(CO)C(=C)C)C	Yes
92	Ledol	CC1CCC2C1C3C(C3(C)C)CCC2(C)O	Yes
93	Limonene	CC1=CCC(CC1)C(=C)C	Yes
94	Linalool	CC(=CCCC(C)(C=C)O)C	Yes
95	Myrcene	CC(=CCCC(=C)C=C)C	Yes
96	Myrcenol	CC(C)(CCCC(=C)C=C)O	Yes
97	Myrtenal	CC1(C2CC=C(C1C2)C=O)C	Yes
98	Myrtenol	CC1(C2CC=C(C1C2)CO)C	Yes
99	n-Heptadecane	CCCCCCCCCCCCCCCCC	Yes
100	n-Hexadecane	CCCCCCCCCCCCCCCC	Yes
101	Neral	CC(=CCCC(=CC=O)C)C	Yes
102	Non-2-en-4-one	CCCCCC(=O)C=CC	Yes
103	Nonan-2-ol	CCCCCCCC(C)O	Yes
104	Nonan-2-one	CCCCCCCC(=O)C	Yes
105	Norbornyl acetate	CC(=O)OC1CC2CCC1C2	Yes
106	Ocimene	CC(=CCC=C(C)C=C)C	Yes
107	Octadecane	CCCCCCCCCCCCCCCCCC	Yes
108	p-Cymen-8-ol	CC1=CC=C(C=C1)C(C)(C)O	Yes
109	p-Cymene	CC1=CC=C(C=C1)C(C)C	Yes
110	p-Menth-1,8-dien-9-ol	CC1=CCC(CC1)C(=C)CO	Yes
111	p-Menth-1-en-9-ol	CC1=CCC(CC1)C(C)CO	Yes
112	p-Menth-4-en-9-ol	CC1CCC(=CC1)C(C)CO	Yes
113	Patchulane	CC1CCC23C1CC(C2(C)C)CCC3C	Yes
114	Perillene	CC(=CCCC1=COC=C1)C	Yes
115	Perillyl alcohol	CC(=C)C1CCC(=CC1)CO	Yes
116	Pinocamphone	CC1C2CC(C2(C)C)CC1=O	Yes
117	Protocatechuic acid	C1=CC(=C(C=C1C(=O)O)O)O	Yes
118	Sabinene	CC(C)C12CCC(=C)C1C2	Yes

Continued

No.	Phytochemical Name	Canonical SMILES	Drug likeness
119	Spathulenol	CC1(C2C1C3C(CCC3(C)O)C(=C)CC2)C	Yes
120	Syringic acid	COC1=CC(=CC(=C1O)OC)C(=O)O	Yes
121	Terpinolene	CC1=CCC(=C(C)C)CC1	Yes
122	Terpinyl acetate	CC1=CCC(CC1)C(C)(C)OC(=O)C	Yes
123	Thymol	CC1=CC(=C(C=C1)C(C)C)O	Yes
124	trans-Dihydroocimene	CC(C)CCC=C(C)C=C	Yes
125	trans-Farnesal	CC(=CCCC(=CCCC(=CC=O)C)C)C	Yes
126	trans-p-Coumaric acid	C1=CC(=CC=C1C=CC(=O)O)O	Yes
127	trans-Pinocarveol	CC1(C2CC1C(=C)C(C2)O)C	Yes
128	trans-Sabinene hydrate	CC(C)C12CCC(C1C2)(C)O	Yes
129	Undecanal	CCCCCCCCCCC=O	Yes
130	Vanillic acid	COC1=C(C=CC(=C1)C(=O)O)O	Yes
131	Zederone	CC1=CCCC2(C(O2)C(=O)C3=C(C1)OC=C3C)C	Yes
132	Zerumbone	CC1=CCC(C=CC(=O)C(=CCC1)C)(C)C	Yes
133	Zerumin A	CC1(CCCC2(C1CCC(=C)C2CC=C(CC(=O)O)C=O)C)C	Yes
134	Zerumin B	CC1(CCCC2(C1CCC(=C)C2CC(C3=CC(OC3=O)O)O)C)C	Yes
135	Zingiberene	CC1=CCC(C=C1)C(C)CCC=C(C)C	Yes

Toxicity Testing Criteria

The assessment of toxicity, quantified by LC50 values, is a widely acknowledged parameter. In accordance with Clarkson's toxicity criteria, extracts are categorized based on their concentrations: extracts with LC50 values exceeding 1000 µg/ml are deemed non-toxic, those with LC50 values ranging from 500 to 1000 µg/ml are considered low toxic, concentrations with LC50 values falling between 100 and 500 µg/ml are classified as medium toxic, and those with LC50 values ranging from 0 to 100 µg/ml are characterized as highly toxic (Clarkson et al., 2004). The LC50 value for the E. foetidum leaf extract is measured at 532.81 µg/ml, placing it within the category of LC50 values ranging from 100 to 500 µg/ml. Consequently, it can be inferred that Curcuma amada exhibits low toxicity.

Results of in silico study

Results of Chemical Profiling and ADMET

In our study, we curated information of 135 compounds from different parts of the *C. amada* that are extracted, elucidated, and reported in many previous studies. The details of these compounds are depicted in Table 4 with their chemical name, SMILE, and drug likeness. Maximum one violation was considered for selection of these compounds.

Table 4. Cavity-detection guided Blind Docking: - (a) Farnesene – Erα prepared (b) 3-Carene – Erα prepared (c) Eupatin – Erα prepared (d) Coumarin – Erα prepared

C. amada	Estrogen receptor alpha (ERα)	(E)-.beta.-Farnesene	-11.3	3174	**Fig- 1, 2**
		3-Carene	-6	3174	Fig- 3,4
		Coumarin	-6.3	5043	Fig- 5,6
		Eupatin	-8.1	5043	Fig- 7,8

Results of Target Protein and Compounds Selection

We selected 4 compounds among these 135 compounds which would be potential for breast cancer treatment (Table 4). We downloaded their 3D structure from PubChem (https://pubchem.ncbi. nlm.nih.gov/) for molecular docking and the selected protein Estrogen receptor alpha (ERα) was downloaded.

Table 5. Vina and Cavity Scores for different docked models

a		b		c		d	
Vina score	Cavity score	Vina score	Cavity score	Vina score	Cavity score	Vina score	Cavity score
-11.33	3174	-6.0	3174	-9.4	440	-8.1	5043
-10.2	5043	-5.8	5043	-6.3	5043	-8.1	3174
-7.9	287	-5.1	440	-6.3	3174	-7.6	396
-7.8	296	-4.8	396	-5.0	396	-7.0	287
-10-6	440	-4.2	287	-4.6	287	-6.0	440

Results of Molecular Docking

The CB-Docking server predicted five docked models for each docking (Table 5). We chose one among the five based on their vina score and cavity size. Here Vina scores indicate the required energy for binding and the Cavity scores indicate the suitable properties for binding with a ligand (https://pubs.acs.org /doi/pdf/10.1021/acs.accounts.5b00516). We took those docked models which have the highest Vina and Cavity Score (Table 6). We also showed the ligand and receptor interaction in figure 4.

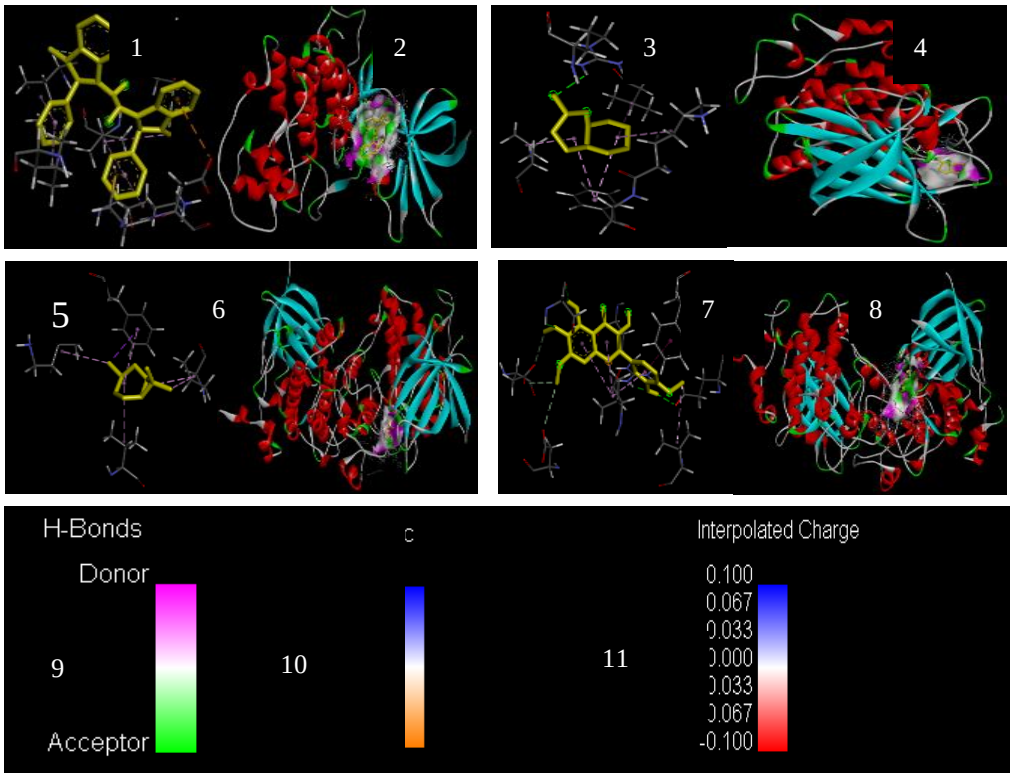

Figure 4. Picture no 1, 3, 5 and 7 are showing the atomic interaction and interpolated charge with the receptor, here the ligand is yellow-colored. Picture no 2, 4, 6 and 8 is indicating the location of the ligand in the docked complex

Discussion

Numerous researchers worldwide have extensively investigated the robust antimicrobial properties inherent in plant extracts (Reddy et al., 2001; Ateb and Erdo, 2003). Effectiveness against diverse pathogens has been demonstrated in the crude extracts derived from medicinal plants (Kubo et al., 1981). An exploration of the antimicrobial potential of the ethanolic rhizome extract of Curcuma amada was conducted, employing the disc diffusion method with various pathogenic microorganisms. The examination revealed that the ethanolic extract from Curcuma amada exhibits both antibacterial and antifungal activities (Chhetri, 2008; Okigbo, 2009).The results of antimicrobial activities of against selected bacterial species such as *Klebsiella pneumonia*, *Escherichia coli*, *Acetobacter aceti*, *and Staphylococcus aureus* are observed through disc diffusion method. In antibacterial studies, extract was most effective against *Escherichia coli* and *Acetobacter aceti* which are gram negative but show no effects on *Klebsiella pneumoniae* and *Staphylococcus aureus* bacterial strains (Table 1).

To assessment the toxicity level of this *Curcuma amada* Rhizome extract, different concentration of rhizome extracts was used such as- 25 ppm, 50 ppm, 100 ppm, 200 ppm. Through the calculation of LC_{50}, the concentration, 100ppm and 200ppm showed most toxicity value. At the mortality rate was observed 10% and 20% at 100ppm and 200ppm, respectively. Depending on the conditions of the experiment been obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (Meyer, Brine shrimp: a convenient general bioassay for active plant constituents, 1982) (Ahmed, 2010; Moshi, 2010). LC_{50} values were estimated using a probit-regression analysis (Table 2). The LC_{50} (Lethal concentration of 50%) value of the *Curcuma amada* on the animal model *Artemia salina* (Brine shrimp). The lower the lethal concentration the higher the toxicity level (Figure 3.3).

Molecular docking is a computational method that assesses the interaction between a ligand and receptor protein at the atomic level, offering insights into the conformation and behavior of small molecules within macromolecules. Chemical profiling furnishes detailed information regarding the indigenous chemical compounds and their properties in a given source. Utilizing virtual screening algorithms, significant chemical compounds can be efficiently identified from a myriad of candidates. In contemporary drug discovery, virtual screening has garnered substantial attention due to its robustness, cost-effectiveness, and time-saving advantages over empirical screening methods.

In the context of our study, four phytochemicals-namely, E)-.beta.-Farnesene, eupatin, 3-Carene, and coumarin-derived from the plant were chosen and subjected to molecular docking against the Estrogen receptor alpha (ERα), a protein implicated in breast cancer. The docking analysis unveiled promising binding scores, with maximum scores of -11.3 (E-.beta.-Farnesene), -6 (eupatin), -6.3 (3-Carene), and -8.1 (coumarin). This suggests the potential bioactivity of these compounds. Consequently, the extract from C. amada holds promise as a prospective drug, offering a safer alternative in its purified form to synthetic chemical-based chemotherapeutics.

Table 6. Selected Compounds and Target Protein Receptor

Plants	Compounds	Canonical SMILES	Compound CID	Target Disease	Target Receptor
C. amada	(E)-.beta.-Farnesene	CC(=CCCC(=CCCC(=C)C=C)C)C	CID:10407	Breast cancer	Estrogen receptor alpha (ERα)
	eupatin	COC1=C(C=C(C=C1)C2=C(C(=O)C3=C(C(=C(C=C3O2)OC)OC)O)O)O	CID:5317287		
	3-Carene	CC1=CCC2C(C1)C2(C)C	CID:26049		

Conclusion

Antimicrobial resistance is a global problem. Hence, this study was aimed to focus the antimicrobial properties of *Curcuma amada* (mango ginger) rhizome extract on some selected bacteria which are gram positive and gram negative. The *Curcuma amada* (mango ginger) rhizome extract shows low level and no observable antimicrobial activity against the selected bacterial strain. So, in case of infection of one of these bacteria, the use of mango ginger powder is not so much effective. The compound exhibits promising bioactive properties with considerable potential for pharmaceutical applications. However, prior to its utilization in humans, it is imperative to conduct essential procedures such as isolating the pure compound, performing toxicological studies, and assessing its pharmacological activity. Further research is warranted to comprehensively evaluate the antimicrobial and cytotoxic effects of mango ginger extracts. Additionally, our investigation revealed the low toxicity of C. armada against A. salina. Furthermore, potential inhibitors of Estrogen Receptor Alpha (ERα) were identified in C. amada. Through virtual screening, four compounds were pinpointed out of 135, and their binding properties were validated via ADMET analysis and Molecular Dynamics (MD) simulations. The MD simulations elucidated crucial binding interactions, showcasing binding scores of -11.3, -6, -6.3, and -8.1 for (E)-.beta.-Farnesene, eupatin, 3-Carene, and coumarin, respectively. These interactions are vital for inhibiting Estrogen Receptor Alpha (ERα) activity. In conclusion, this study lays the groundwork for developing novel drugs that inhibit Estrogen Receptor Alpha (ERα) to combat breast cancer, leveraging the natural source of C. amada.

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Conflict of Interests

The authors have no disclosure to make that qualifies as a conflict of interest.

References

Ahmed, Y., Sohrab, M. H., Al-Reza, S. M., Tareq, F. S., Hasan, C. M., &Sattar, M. A. (2010). Antimicrobial and cytotoxic constituents from leaves of *Sapium baccatum*. *Food Chem. Toxicol.* ***48*****(2):** 549-552.

Alzoreky, N. S., & Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int.l J.l Food Microbiol.* ***80*****(3):** 223-230.

Anand, P., Thomas, S. G., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Sung, B., ... & Aggarwal, B. B. (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem. Pharmacol.* ***76*****(11):** 1590-1611

Apisariyakul, A., Vanittanakom, N., & Buddhasukh, D. (1995). Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). *J. Ethnopharmacol.* ***49*****(3):** 163-169.

Ateş, D. A., & turgay, Ö. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. Biol.* ***27*****(3):** 157-162.

Bartz, J. A., & Brecht, J. K. (Eds.). (2002). *Postharvest physiology and pathology of vegetables* (Vol. 123). Crc Press.

Chandarana, H., Baluja, S., & Chanda, S. (2005). Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. *Turk. J. Biol.* ***29*****(2):** 83-97.

Chhetri, H. P., Yogol, N. S., Sherchan, J., Anupa, K. C., Mansoor, S., &Thapa, P. (2008). Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kath. Univ. J. Sci. Eng. Technol.* ***4*****(1):** 49-54.

Ibrahim, H. M., & Abu-Salem, F. M. (2015). Antibacterial activity of some medicinal plant extracts. *Int J. Nutr. Food Eng*. ***8*****(10):** 1168-1173.

Islam, M. (2004). *Genetic diversity of the genus Curcuma in Bangladesh and further biotechnological approaches for in vitro regeneration and long-term conservation of C. longa germplasm* (Doctoral dissertation, Hannover: Universität).

Islam, M. R., Ahamed, R., Rahman, M. O., Akbar, M. A., Al-Amin, M., Alam, K. D., &Lyzu, F. (2010).

In vitro antimicrobial activities of four medicinally important plants in Bangladesh. *Eur. J. Sci. Res*. ***39(2):***199-206.

Kubo, M., Kimura, Y., Shin, H., Haneda, T., Tani, T., &Namba, K. (1981). Studies on the antifungal substance of crude drug: 2. On the roots of Polygonumcuspidatum (Poligonaceae). *ShoyakugakuZasshe*, ***35*(1):** 58-61.

Lamo, J. M., & Rao, M. H. S. R. Morpho-Genetic Diversity between and within the Species of the Genus *Curcuma* L and its Relevance for Conservation Efforts. *Biol. Biotechnol. Sustain. Dev.* 197.

Madhuri, S., & Pandey, G. (2009). Some anticancer medicinal plants of foreign origin. *Curr. Sci.* 779-783.

Mangunwardoyo, W., & Usia, T. (2012). Antimicrobial and identification of active compound *Curcuma xanthorrhiza* Roxb. *Med. Env. Sci.* ***12((2)***

Moshi, M. J., Innocent, E., Magadula, J. J., Otieno, D. F., Weisheit, A., Mbabazi, P. K., &Nondo, R. S. O. (2010). Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, northwestern Tanzania. *Tanzan. J Health Res*. ***12*(1):** 63-67.

Nasar-Abbas, S. M., & Halkman, A. K. (2004). Antimicrobial effect of water extract of sumac (Rhuscoriaria L.) on the growth of some food borne bacteria including pathogens. *Int. J. Food Microbiol.* ***97*(1):** 63-69.

Okigbo, R. N., Anuagasi, C. L., Amadi, J. E., &Ukpabi, U. J. (2009). Potential inhibitory effects of some African tuberous plant extracts on Escherichia coli, Staphylococcus aureus and Candida albicans. *Int. J. Integr. Biol.* ***6*(2):** 91-98.

Policegoudra, R. S., & Aradhya, S. M. (2008). Structure and biochemical properties of starch from an unconventional source—Mango ginger (*Curcuma amada* Roxb.) rhizome. *Food Hydrocol.* ***22*(4):** 513-519.

Policegoudra, R. S., Abiraj, K., Gowda, D. C., & Aradhya, S. M. (2007). Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. *J. Chromatogr. B*. ***852*(1-2):** 40-48.

Policegoudra, R. S., Divakar, S., &Aradhya, S. M. (2007). Identification of difurocumenonol, a new antimicrobial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. *J. Appl. Microbiol*. ***102*(6):** 1594-1602.

Revathy, S., Elumalai, S., & Antony, M. B. (2011). Isolation, purification and identification of curcuminoids from turmeric (Curcuma longa L.) by column chromatography. *J. Exp. Sci.* ***2*(7).**

Salvi, N. D., George, L., & Eapen, S. (2000). Direct regeneration of shoots from immature inflorescence cultures of turmeric. *Plant Cell Tissue Organ Cult.* ***62*(3):** 235-238.

Sasikumar, B. (2005). Genetic resources of Curcuma: diversity, characterization and utilization. *Plant Genet. Resour.* ***3*(2):** 230-251.

Shankaracharya, N. B. (1982). Mango ginger. *Indian Cocoa, Arecanut Spices J.* ***5*(4):** 78-80.

Shirgurkar, M. V., John, C. K., & Nadgauda, R. S. (2001). Factors affecting in vitro microrhizome production in turmeric. *Plant Cell Tissue Organ Cult.* ***64*(1):** 5-11.

Siddaraju, M. N., &Dharmesh, S. M. (2007). Inhibition of gastric H+, K+-ATPase and Helicobacter pylori growth by phenolic antioxidants of Zingiberofficinale. *Mol. Nutr. Food Res*. ***51*(3):** 324-332.

Srinivasa Reddy, P., Jamil, K., Madhusudhan, P., Anjani, G., & Das, B. (2001). Antibacterial activity of isolates from Piper longum and Taxusbaccata. *Pharm. Biol.* ***39*(3):** 236-238.

Srinivasan, D., Nathan, S., Suresh, T., & Perumalsamy, P. L. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol*. ***74*(3):** 217-220.

Warrier, P. K., Nambiar, V. P. K., &Ramankutty, C. (1994). Indian medical plants.

Yoshioka, T., Fujii, E., Endo, M., Wada, K., Tokunaga, Y., Shiba, N., & Muraki, T. (1998). Anti-inflammatory potency of dehydrocurdione, a zedoary-derived sesquiterpene. *Inflamm. Res.* ***47* (12):** 476-481.