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In vitro Antioxidant and Antibacterial Activity and Phytochemical Screening of Mango Ginger (*Curcuma amada* Roxb.) in Mizoram

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ABSTRACT: Mango ginger (*Curcuma amada* Roxb.) is a perennial, rhizomatous, fragrant herb, having morphological resemblance with ginger but imparts a raw mango flavour. This plant has been utilised for the treatment of many ailments in traditional medical systems (Ayurveda and Unani) from ancient times. *Curcuma amada* possesses several pharmaceutical properties such as antimicrobial, anti-inflammatory, analgesic, anticancer, anti-hyperglyceridemic, antioxidant activity etc. Owing to these properties, an experiment was conducted at Mizoram University, Aizawl (2021), to evaluate the phytochemical constituents of *Curcuma amada* Roxb. (Zingiberaceae). The study revealed the potential antioxidant and radical scavenging activity (IC_{50} 223.2 µg/ml) of *C. amada* rhizome extracts by DPPH assay, indicating its protective role against oxidative damage and as an important natural antioxidant. The above said pharmaceutical activity may be shown due to the presences of various bioactive compounds (screened using respective scientific methods and protocols) including phenols (11.47±0.004 mg/100 mg), flavonoids (5.05±0.068 mg/100mg), ascorbic acid (5.05± 0.068 mg/100mg), tannins, saponin, glycosides etc. The antibacterial activity, determined by the disc diffusion assay, of *C. amada* rhizome extract exhibits an inhibition zone of 12±0.2 mm, 11±0.5 mm, 10±0.2 and 8.6±0.7 mm diameter against *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* culture respectively. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Curcuma amada*, mango ginger, phytochemical screening, quantitative analysis, antioxidant activity, antibacterial activity.

INTRODUCTION

Plants are regarded as living biochemical factories that produce a wide range of chemical molecules known as phytochemicals or secondary metabolites with different biological activities as a result of their metabolic processes (Kaur *et al*., 2018; Mahadevi & Kavitha, 2020) and these phytochemical constituents occur in different plant parts such as leaf, stem, root, flower, bark etc. (Gordon, 2001). Primary constituents include carbohydrates, amino acids, proteins and chlorophyll, whereas secondary metabolites include alkaloids, terpenoids, steroids, flavonoids, saponins etc. (Dhawale, 2003). About 80% of the world's population rely on traditional medicine that involves use of plant and herb extracts for their primary healthcare system (Sandhya *et al*., 2006). Due to their wide availability and fewer side effects, herbal medicines are acceptable for treating a broad range of infection and diseases (Chattopadhyay *et al*., 2004). *Curcuma* genus of the Zingiberaceae family consists of about 80 species, of which 40 are indigenous to India. Extensive research has been conducted on *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger), but the medicinal properties of *Curcuma amada* (mango ginger) has yet to be fully explored (Saipriya, *et al*., 2017). *Curcuma amada* originated in the Indo-Malayan region and widely distributed in the tropics from Asia to Africa and Australia (Sasikumar, 2005) with a geographical distribution ranging from India, Indo-China, Thailand, Indonesia, Malaysia and northern Australia (Policegoudra *et al*., 2011). *C. amada* is found in the wet (semi-evergreen) mixed forests of West Bengal (Mallick, 2019), and is cultivated in the North Eastern states, Gujarat, Uttar Pradesh, Kerala, Karnataka and Tamil Nadu (Policegoudra *et al*., 2011). *C.amada* is an aromatic herb known as Amba haldi or Mango ginger. It closely resembles the morphology of ginger; its rhizome gives a characteristic odour of raw mango flavour due to the presence of terpene hydrocarbons cis-ocimene and car-3-ene which makes mango ginger a unique spice (Gholap *et al*., 1984). It is used as a major ingredient in pickles, candies, salads, sauces and chutneys (Yogamaya *et al*., 2012). Therapeutically, it is used to treat a range of mood and medical disorders in traditional and Ayurvedic medicine (Policegoudra *et al*., 2011). *Curcuma* plants have a camphoraceous aroma and contain many functional compounds such as phenolics, flavonoids and different antioxidant enzymes (Krishnaraj *et al*., 2010) and various species belonging

to this genus are well known for their multiple use as medicine, cosmetic, dye, flavouring agent and nutraceuticals. Likewise, the antioxidant and anti-inflammatory activities of *C. amada* is due to the presence of phenolic compounds (Mara *et al.*, 2006). The essential oil of rhizome exhibits antimicrobial, antifungal and anthelmintic activity against tape worms and such pharmaceutical properties may be shown due to the presences of various bioactive compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, phenol and terpenoids (Policegoudra *et al*., 2011). *Curcuma amada* rhizomes are exported from India as medicinal plant parts (Hasan *et al*., 2009). Few considerable studies have been conducted on *C. amada* regarding its phytochemicals as well as further screening of the found constituents. Although there has been some documentation on the traditional uses and its benefits yet the indigenous knowledge on the various aspects of usage has been passed down from generation to generation orally. There is a need for the proper documentation and elaborate study on this plant species. Huge potential is observed in future studies and research which can be carried out with respect to the fields of medicine, pharmaceutical, pharmacology, oil industries, food industries, perfumery etc. which in the long run will not only elevate better treatment options for ailments but also give an immaculate area for economic growth of the local people with proper cultivation practices and better marketing approaches. Therefore, this study aims at understanding and analysing the primary photochemical constituents of *C. amada*.

MATERIAL AND METHODS

Collection of plant material: *C. amada* rhizomes were collected from Amtali village, Takarjala, West Tripura during March, 2021. The rhizome samples were thoroughly cleaned under tap water. Fresh rhizomes of *C. amada* were used for further experiments.

Phytochemical screening: Standard phytochemical screening protocols were used to detect the presence of bioactive agents. These tests were identified by visual inspection of colour changes or precipitate formation after the addition of particular reagents to the solution.

Test for Tannin (Braymer's Test): 2 ml of extract was mixed with few drops of 5% $FeCl_2$ solution and blue colour was observed to indicate the presence of tannins.

Test for Saponin (Foam Test): 2 ml of extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously. Formation of stable foam was observed to indicate the presence of saponin.

Test for Flavonoid (NaOH Test): 2 ml of extract was added into 2 ml of 10% NaOH solution. Yellow to orange colour was observed to indicate the presence of flavonoids.

Test for Protein (Xanthoproteic Test): 2 ml of extract was added into 2 ml of HNO_3 and boiled in water bath. Orange colour was observed to indicate the presence of protein.

Test for Carbohydrate (Benedict's Test): 2 ml of extract was mixed with 2 ml of Benedict's reagent and boiled in water bath. Yellow, green or red precipitate was observed to indicate the presence of carbohydrate.

Test for Glycosides (Keller-Kiliani Test): 2 ml of extract was mixed with 2 ml of glacial acetic acid containing 2 drops of 2 % $FeCl_2$ solution. The mixture was poured into another test tube containing 2 ml of concentrated H_2SO_4. Brown ring at the interface was observed to indicate the presence of cardiac glycosides.

Quantitative analysis: Determination of Flavonoid content: To determine the total flavonoid content, $AlCl_2$ method was used. 1g of fresh sample was weighed and crushed by mortar and pestle. 0.3 ml of $NaNO_2$ and 4 ml of H_2O was added and kept for 5 minutes. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added and left for 6 minutes. At 6 minutes, 2 ml of 1M NaOH was added and volume was made up to 10 ml with distilled water. The absorbance was recorded at 510 nm using a digital spectrophotometer.

Determination of Carbohydrate content: Total carbohydrate was determined by using Anthrone reagent and HCL following the Anthrone method and absorbance was measured using a digital spectrophotometer at 630 nm.

Determination of antioxidant content: Total antioxidant was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) and methanol following DPPH assay and absorbance was measured using a digital spectrophotometer at 517 nm. The free radical scavenging activity (percentage antiradical activity) was calculated by the equation

$$\% \text{ antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Determination of Phenol Content: The total phenolic content was determined according to Folin-Ciocalteu method (FCM) described by Siddhuraju and Becker, 2003 using catechol as a standard. Different aliquots (0.5 & 1 ml) are taken in test tubes diluted in 3 ml of water and 0.5 ml of Folin-Ciocalteu reagent is added. After 3 minutes, 2 ml of 20% Na_2CO_3 is added in each tube and mixed the content thoroughly. Colour was developed and absorbance was measured at 650 nm using digital spectrophotometer against blank reagent. Standard graph was prepared using different concentrations of catechol.

Determination of Protein content: The total protein content was determined by Folin Lowry Method using bovine serum albumin as standard. The absorbance was measured at 660 nm using a digital spectrophotometer.

Determination of Ascorbic acid: The total ascorbic acid content was determined by Volumetric method using reagent 2,6-dichlorophenolindophenol and the result is expressed in mg/100 ml sample.

Determination of Anthocyanin content: Anthocyanin was estimated by taking a gram of sample and adding 50-60 ml of Methanolic HCl (85:15v/v). The produced extract was stored for a day under airtight condition and then diluted to 100 ml with Methanolic HCl. The colour

density was measured using a digital spectrophotometer at 445 nm.

Antibacterial studies: Evaluation of antibacterial activity of *C. amada* rhizome extract was carried out by disc diffusion assay described by Lennette, 1985. The rhizome extracts were diluted with Dimethyl sulphoxide (DMSO) and aliquots were loaded on a disc and the antibacterial activity was evaluated against five standard bacterial strains which included the Gram positive *Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC-441) and Gram negative *Pseudomonas aeruginosa* (ATCC-15442), *Bacillus Pumilus* (ATCC-14884) & *E. coli*. The bacterial strains were inoculated on freshly prepared agar plates with a loop, evenly spread by a spreader and incubated overnight at 37°C. The diameter of zone of inhibition produced by the inoculums were measured in mm.

RESULT AND DISCUSSION

Phytochemical analysis revealed the presence of tannin, saponin, glycoside, protein, carbohydrate and flavonoid. Saponin is a natural antioxidant which also promotes tumour cell death (Podolak *et al*., 2010; Tapondjou *et al*., 2011; Bi *et al*., 2012). Saponins have anti-hypercholesterolemic activities as well as antibacterial characteristics. Tannin has been employed as an active ingredient in medicine and beverages due to its antioxidant properties (Amarowicz and Troszynska, 2003; Amarowicz *et al*., 2005). Tannins have been shown to prevent the growth of harmful fungus. Strong lipid peroxidation inhibitors have been found in glycosides such as quercetin monoglycosides, diglycosides and flavonol glycosides (Plumb *et al*., 1999). Physiochemical parameters of the rhizome of *Curcuma amada* Roxb. are tabulated in Table 1.

Table 1: Biochemical constituent of *Curcuma amada.*

Parameters	Sample result
Tannin	+
Saponin	+
Glycoside	+
Protein	+
Carbohydrate	+
Flavonoid	+

Antioxidant activity: Plants having antioxidant and radical scavenging properties are valuable in medical applications and pharmaceutical industries. The antioxidant capacity of *C. amada* was assessed in this work using the DPPH radical scavenging method and compared to the activity of ascorbic acid, a well known antioxidant (Fig. 1). The different concentrations of 50, 100, 150, 200, 250 and 300 µg/ml showed different levels of radical scavenging activity of 17.7, 26.0, 29.1, 38.5, 53.1 and 73.9 % of inhibition respectively with an IC_{50} value of 223.2 µg/ml while ascorbic acid content has an IC_{50} value of 107.5µg/ml.

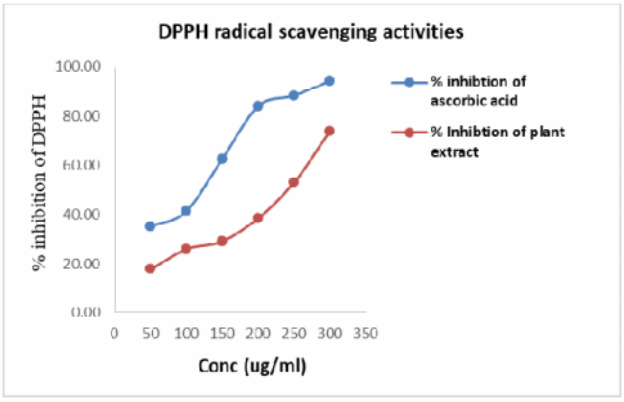

Fig. 1. Inhibition % of ascorbic acid and plant extract.

Total flavonoid content: The extracts' total flavonoid content was calculated as a percentage of quercetin equivalents per 100 mg of the sample (Fig. 2). The total flavonoid estimation of rhizomes of *C. amada* showed the content value of 5.05± 0.068 mg/100mg.

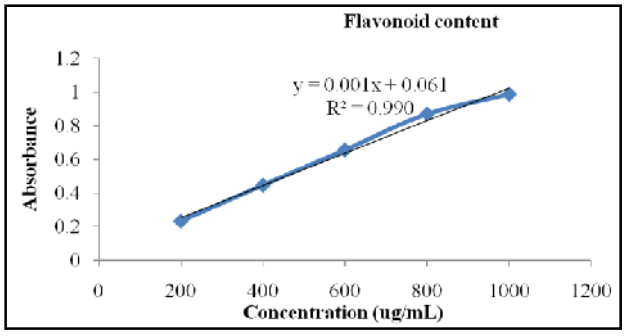

Fig. 2. Standard graph of flavonoid content.

Total phenol content: The total phenol estimation of *C. amada* rhizomes showed the content value of 11.47±0.004 mg/100 mg (Fig. 3).

Total protein content: The total protein content of the rhizomes of *C. amada* showed the content value of 12.47 ± 0.03 mg/g (Fig. 4).

Total carbohydrate content: The total carbohydrate content of *C. amada* rhizomes showed the value of 14.55 ± 0.06 mg/100mg (Fig. 5).

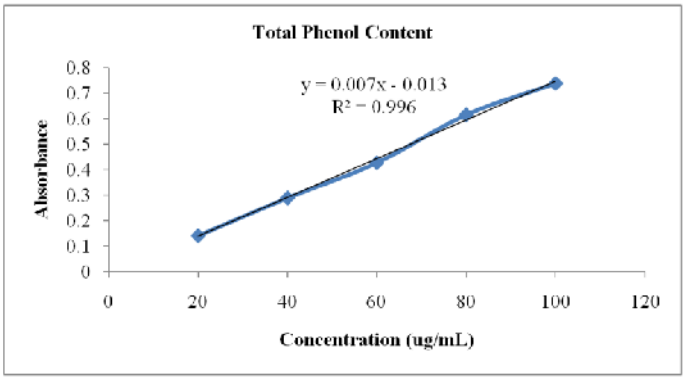

Fig. 3. Standard graph of phenol content.

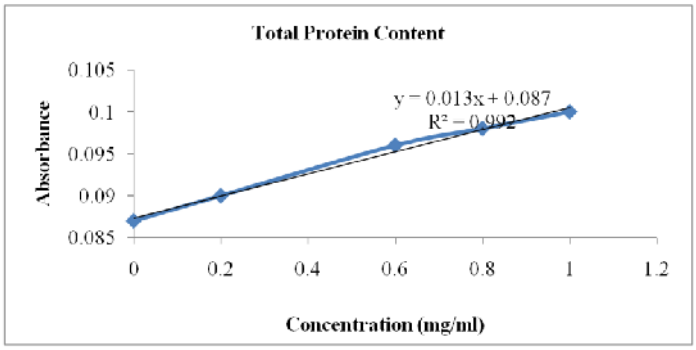

Fig. 4. Standard graph of protein content.

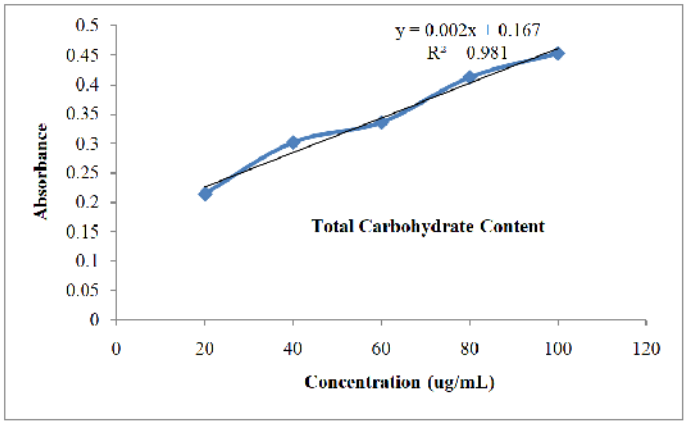

Fig. 5. Standard graph of carbohydrate content

Total anthocyanin content: The total anthocyanin content of *C. amada* rhizome showed a value of 5.29 ±0.001 mg/100g**.**

Total ascorbic acid content: The total ascorbic acid content of rhizome of *C. amada* showed a content value of 5.05± 0.068 mg/100mg.

Antibacterial activity: The antibacterial activity of the extracts varied depending on the extract concentration and was measured in terms of diameter, is shown in Table 2. The maximum inhibition zone observed was 12 mm against bacteria *Escherichia coli* followed by 11 mm and 10 mm against *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively and the minimum inhibition observed was 8.6 mm against *Bacillus subtilis.*

Table 2: Zone inhibited by *C. amada* against gram +ve & gram -ve bacteria strains.

Sr. No.	Bacteria	Inhibition zone (mm)	Std (±)
1	*Escherichia coli*	12	0.2
2	*Pseudomonas aeruginosa* (ATCC-15442) -ve	11	0.5
3	*Staphylococcus aureus* (MTCC-96) +ve	10	0.2
4	*Bacillus subtilis* (MTCC-44) +ve	8.6	0.7

Spices are economically significant and high in phenolic chemicals and flavonoids, which are easily absorbed by our body and does not cause harm (Chandarana *et al.*, 2005). In the present study we have observed that *Curcuma amada* extract contains carbohydrate, flavonoid, phenol, tannin, anthocyanin and glycoside compounds, all of which are known to have therapeutic effects against disease causing microorganisms. The findings suggest that the rhizome of *Curcuma amada* holds promise as a potent source of pharmaceutically important compounds. The most major classes of secondary metabolites and bioactive substances found in plants are flavonoids and phenolic compounds (Surapaneni and Vishnu 2009). Flavonoids found in the non-aerial portions of plants, such as rhizome, plays an important function in metabolism and development in living systems. Phenolic compounds are a type of antioxidant agents that operate as free radical terminators. Their bioactivities may be connected to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Roya & Fatemeh, 2013). The study has revealed the total phenolic and flavonoid content of 11.47 and 5.05 mg/100 mg respectively which gives them their antioxidant properties**.** Earlier reports have shown that some flavonoids, such as quercetin are anticarcinogenic and can stop cancer cells from growing (Elattar & Virji, 2000; Ranellett *et al.*, 1999).

CONCLUSION

The study revealed the potential antibacterial, antioxidant and radical scavenging activity of extracts of *Curcuma amada* rhizomes, indicating its protective role against microbial infections, oxidative damage and as an important natural antioxidant. *C. amada* possesses several pharmaceutical properties such as antimicrobial, anti-inflammatory, analgesic, anticancer, anti-hyperglyceridemic, antioxidant activity etc. The above said pharmaceutical activity may be shown due to the presences of various bioactive compounds including tannins, saponin, flavonoids, phenolics, glycosides etc. It may be stated that the phytochemical examination provided valuable information about the various phytoconstituents found in the plant, which will aid future researchers in selecting the appropriate extract for further exploration of isolating the active principle.

FUTURE SCOPE

The varied phytochemical and biological activities of *C. amada* reported in the present study may confirm the therapeutic value, for its combating abilities and use against multiple diseases. Further, it may be scientific validation for bioactive properties of mango ginger rhizome and its usage in Ayurveda and other traditional medicines. However, the structure–activity relationships and pharmacological activity of these constituents is the need of the hour. Further synthesis of active principles can lead to development of pharmacological products for health benefits. The present investigation was mainly confined to analyse and understand the primary photochemical constituents of *C. amada*. So, further phytochemical investigation on anticarcinogenic compounds from the plant is to be imitated. To find more effective response, regarding the isolation, characterization and to elucidate the structure of the bioactive compounds of this plant for antimicrobial drug formulation, experiment study should be designed in such a way to develop an economically viable drug for pharmaceutical industry as well as in traditional medicine. Since the findings are based on the observation of one species of *Curcuma viz*., *Curcuma amada*, the experiment should be repeated and continued for different species of *Curcuma* for better interpretation of results.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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