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Investigating the phytochemical, antibacterial, antifungal, and insecticidal properties of *Curcuma Amada* Roxb rhizome extracts

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Abstract
The present investigation was focused on the study of the chemical composition variability and biological activities of the rhizome extracts from *Curcuma amada* Roxb. The separation of the constituents was carried out by gas chromatography coupled with mass spectrometry (GC-MS). The *C. amada* rhizome extract was characterized by a high content of sesquiterpenes such as Curcumene (b,g). An antibacterial activity of rhizome extract from *C. amada* was tested against *Xanthomonas oryzae pv oryzicola* and *Xanthomonas campestris pv oryzae*. We also examined the antifungal activities against *Rhizoctonia solani*, and *Magnaporthe oryzae*. Further, we evaluated the pesticidal efficacy of *C. amada* rhizome extract against *Nilaparvata lugens*. All tested samples displayed noteworthy antibacterial antifungal properties, with the highest activity observed on *Xanthomonas oryzae pv oryzicola* and *Rhizoctonia solani*. While, the pesticidal action was remarkably observed on *Nilaparvata lugens* with 67.0% mortality after 72 h of exposure. In conclusion, this study showed pronounced role of the *C. amada* rhizome extract as antibacterial, antifungal and insecticidal activity.

Keywords: Antibacterial, antifungal, *C. amada*, insecticidal, medicinal plant, rhizome

Introduction
The extensive and indiscriminate use of these synthetic chemicals against pests eventually gained resistance. Owing to the hazards of chemical pest control, alternative approaches that are equally effective could be employed to eradicate these pests without compromising human health (Parr, 2015; Manchikanti, 2019) [20, 15]. Alternative to synthetic pesticides might arise from natural and organic plant-derived chemicals. In this regard, recent studies have discovered several intriguing plant extracts that naturally repel insects (Choy *et al* 2014) [8]. These plant extracts have the potential to control pest as they concurrently serve as insecticides and deter insects by preventing them from feeding and laying eggs (Sarwar, 2015) [24]. The chemical composition of medicinal plants contains various secondary metabolites and essential oils that are generated impact the mechanisms of action and establish the economic and medical value of different plants. *Curcuma sp.* is also well known for its insecticidal characteristics, which assist it to fight against a variety of household and agricultural insect pests. Additionally, it exhibits some insect-repelling qualities and the active components of *Curcuma* are an inexpensive and safe substitute for synthetic pesticides, as demonstrated by experts. It was critical to evaluate their efficacy in relation to other readily available plant species. Therefore, it is imperative to evaluate their efficacy with other readily available plant species (Wang and Shelomi, 2017) [31]. Keeping in view of this, we aimed at evaluating the *C. amada* rhizome extract components to combat a variety of plant diseases, including pests, fungi, and bacteria.
Zingiberaceae family includes the valuable aromatic medicinal herb *Curcuma amada* Roxb. Because of its raw mango flavor in the rhizome (Tamta *et al*., 2016, Akter *et al*., 2019) [28, 2] this plant is also known as "Amba ada" or "Mango ginger" (Sutar *et al*., 2020) [26]. This plant is useful for food, medicine, and industry. It also smells good. It has long been used in Indian traditional medical systems, such as Ayurveda and Unani, to treat a variety of conditions, including cancer, tumors, asthma, bronchitis, alexiteric eruptions, skin disorders, coughing fits, and inflammation brought on by trauma (Rajkumari and Sanatombi, 2017, Sutar *et al*., 2020) [22, 26].
Very little information has been found till now regarding the application of *C. amada* rhizome extracts in the prevention and treatment of plant diseases. In light of this, the data we presented is unique, which emphasizes on the antifungal, antibacterial, and pesticidal properties of *C. amada* extracts. This certainly would improve the agrochemical sector's understanding of the specific role this medicinal plant serves in safeguarding both the environment as well as human health.

Materials and methods
Preparation of plant extracts
C. amada was collected and the harvested plants were identified and rhizomes were seperated. Matured rhizomes were air-dried for 72 h, and plant extracts were obtained by magnetic stirring of 2.5 g of powdered dry matter with 25 mL of solvent for 30 min at room temperature (25◦C). Extracts were obtained with solvents of increasing polarity: hexane/water (8:2, v/v), acetone/water (8:2, v/v) ethanol/water (9:1, v/v), methanol/water (8:2, v/v) and water. The extracts were kept for 24 h at 4◦C, filtered through Whatman No. 4 filter paper, evaporated to dryness under vacuum and stored at 4◦C until analysis.

Estimation of total phenolics content
Total phenols were assayed according to Dewanto *et al*. (2002) [9]. An aliquot of diluted extract was added to 0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 mL of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark, absorbance at 760 nm was read versus a prepared blank. The

total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) from a calibration curve with gallic acid. All samples were analyzed in three replicates.

Estimation of total flavonoids

Estimation of total flavonoid content was carried out with the help of aluminium chloride method given by Dewanto *et al*. (2002) [9]. Total 250 mg of powdered sample was suspended in 10 ml of 80% methanol for 24 h at room temperature followed by centrifugation at 3000 rpm for 20 min. 1.0 ml of supernatant extract was taken out in the test tubes. To this, 0.3 ml of 5% $NaNO_2$ solution was added and was left it for 3 min. After 3 min, 0.3 ml of 10% $AlCl_3$ was added and was left the solution for 2 min. 2 ml of 1.0 M NaOH was added and mixed well. After that 2.4 ml distilled water was added to it and mixed well. Optical density of the yellowish brown color was read at 510 nm. Total flavonoid content was calculated from the standard curve prepared from the querctine and the results were expressed in mg/g dry weight.

Total condensed tannins

Condensed tannins (proanthocyanidins) were determined according to the method of Sun *et al*. (1998) [25]. To 50 µL of diluted sample, 3 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 15 min, and absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW. All samples were analyzed in triplicate.

DPPH assay

The electron donating ability of the obtained extracts was measured by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Hanato *et al*. (1988). Extracts (0.1 mL, 5, 10, 50 and 100 mg/mL) were added to 0.5 mL of 0.2 m mol/L DPPH–methanol solution. After incubation for 30 min at room temperature, the absorbance was determined against a blank at 517 nm. The percentage inhibition of free radical DPPH was calculated from (A blank−A sample/A blank) × 100, where *A* blank is the absorbance of the control reaction and *A* sample is the absorbance in the presence of plant extract. The concentration of extract that caused 50% inhibition (IC50) was calculated from the regression equation for the concentration of extract and percentage inhibition. Samples were analyzed in triplicate.

Total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate–Mo(V) complex at acid pH Prieto *et al*, (1999) [21]. An aliquot (0.1 mL) of plant extract was added to 1 mL of reagent solution (0.6 mol/L H_2SO_4, 28 mmol/L Na_3PO_4 and 4 mmol/L ammonium molybdate). The tubes were incubated in a thermal block at 95◦C for 90 min. Once the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. Antioxidant capacity was expressed was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0–500 mg/mL. All samples were analyzed in triplicate.

Gas chromatography/mass spectrometry (GC/MS) analysis

The composition of *C. amada* rhizome was analyzed using SHIMADZU GCMSQP2010. The column used was 30 m × 0.25 mm inner diameter with a thickness of 0.25 µm. The carrier gas used was Helium gas (flow rate of 1 mL/min). The injector temperature was set at 280 ◦C and the oven temperature was raised to 300 ◦C at a rate of 4 ◦C per minute. The identification was carried out using the NIST library according to the standard methods described in the previous reports of Padalia *et al*. (2013) [18].

Collection of microorganisms

Antibacterial activity was determined against major rice plant pathogenic bacteria (*Xanthomonas oryzae pv oryzicola* and *Xanthomonas campestris pv oryzae*). The antifungal screening was carried out against pathogenic fungi (*Rhizoctonia solani* and *Magnaporthe oryzae*) of rice plant. All these organisms were collected from the Crop Protection and Biological Control Laboratory, SLN Biologicals LLP, Nizamabad, Telangana, India.

Collection of test insect

For insecticidal screening the brown plant hopper *(Nilaparvata lugens)* used in the experiment was provided from the stock cultures of the Crop Protection and Biological Control Laboratory, SLN Biologicals LLP, Nizamabad, Telangana, India.

Growth media and conditions

Nutrient agar media (HiMedia Laboratories) pH 7.2 and Potato dextrose agar media (HiMedia Laboratories) pH 5.6 were used for antibacterial and antifungal screening respectively.

Antibacterial screening

The *in vitro* antibacterial activity of the extract was determined by disc diffusion method (Bauer *et al.* 1996) [6]. Sample discs were prepared by allowing each sterile disc (6 mm in diameter) of filter paper to absorb 20 µl of a test solution in aseptic condition. The discs were allowed to dry until complete evaporation of solvent. Dried and sterilized filter paper discs, each containing a test sample of 500 µg of the test agent was placed on nutrient agar medium uniformly seeded with the test microorganisms. Kanamycin disc (30 µg/disc) and blank disc were used as the positive and negative control respectively. The plates were incubated at 37°C for 24 hours for optimum growth of the organisms. The antibacterial activity of the extract was determined by measuring the diameter of the zone of inhibition expressed in millimeter.

Antifungal screening

The extract was screened for its antifungal activity by disc diffusion method at the concentration of 500µg/disc. Potato dextrose (20 ml) plates were prepared and incubated by spread plate method under aseptic conditions. The sterile impregnated discs with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with agar surface. Control discs of carbendezim were prepared and placed on the agar surface. All the plates were incubated at 37°C for 72 hours and the size of the inhibition zones were measured. The mean zone of inhibition of the three replicated tests

(triplicate analysis) of the plant extract is expressed in millimeter.

Insecticidal screening

The experiment for mass rearing of *Nilaparvata lugens* was carried out in a cage set up at growth chamber of Entomology Unit, SLN Biologicals Laboratory, Nizamabad, Telangana, India. The growth chamber was maintained under standard controlled conditions at 25ºC ± 2, RH 65 to 75% with a photoperiod of 12h light and 12h dark (Padmakumari *et al*., 2013; Anusha *et al*., 2022) [19, 5]. The adult insects were collected from the rice fields and released onto the potted rice plants kept in a cage for oviposition. The leaves harboring egg mass were detached from plant and placed in glass tube containing wet cotton ball and plugged with dry cotton for proper aeration. The larvae generated from eggs were allowed to grow in a glass tube containing fresh stems immersed in standardized artificial diet with varying ingredients as food source (5% sucrose). The stems were frequently replaced with fresh ones in order to avoid to decomposition. The mixed population of adult insects was again transferred to potted rice plants for oviposit and the cycle continued for 2 to 3 generations. The plant extract (500 mg) was dissolved into 1 ml methanol. This was poured into the lower part of the glass tube. A control experiment applying only the solvent into the glass tube was also set at the same time under the same conditions

Statistical analysis

Data were analyzed by analysis of variance (ANOVA), and differences among treatments were assessed using Duncan's multiple range test (DMRT) at the $P = 0.05$ level using SigmaPlot version 12.0. Probability analysis was performed for the calculation of LC50 values using the EPA Probit analysis program version 1.5. Inhibition ratio and EC50 values were obtained using Log-Probit analysis.

Results

Total phenolic, flavonoid and tannin contents

In the given study the Fig. 1 illustrates the extracts' total phenol contents. When extracted in methanolic solvents (55 mg GAE/g DW), *C. amada* rhizome extract had a greater total polyphenol content than when extracted in other solvents (Fig. 1a). The highest quantity was found in the methanol extract, which was followed by the extracts of acetone, ethanol, hexane, and water; similar pattern was observed for the levels of flavonoids and condensed tannins (except hexane exhibiting least) (Fig. 1b & 1c).

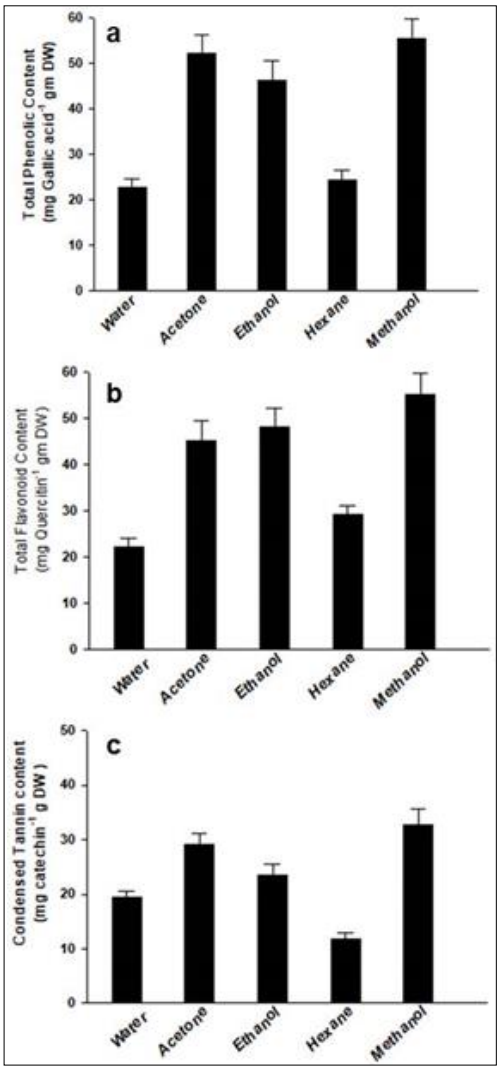

Fig 1: The total contents of (a) polyphenol (expressed as mg gallic acid equivalents/g dry weight), (b) flavonoids and (c) condensed tannin (expressed as mg catechin/g dry weight) in rhizome extracts of *C. amada* in different solvents

Total antioxidant activity and DPPH radical-scavenging activity

The total antioxidant capacity of *C. amada* rhizome extract varied according to the solvent. Our results depicted that total antioxidant capacity of *C. amada* rhizome extract was much higher in methanol extract, followed by the ethanol extract, the acetone extract, the extract in water and least was observed in the hexane extract (Fig 2a).

Even the DPPH radical-scavenging activity (Fig 2b) exhibited similar pattern as that of total antioxidant capacity. The rhizome extracts of *C. amada* extracted in methanol exhibited significantly high DPPH radical scavenging activity than those collected in other solvents (Fig. 2b). Water extracts had the lowest anti-radical activity, while methanol had potent activity followed by ethanol and acetone.

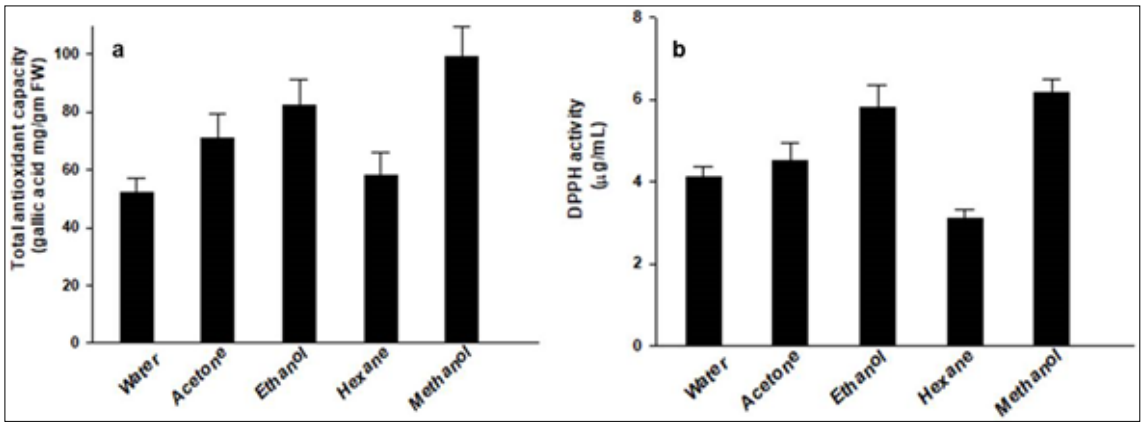

Fig 2: (a) Total antioxidant capacity (expressed as mg gallic acid equivalents/g dry weight and (b) Scavenging activity, expressed as median inhibitory concentration (mg/mL), in the DPPH test in rhizome extracts of *C. amada* in different solvents

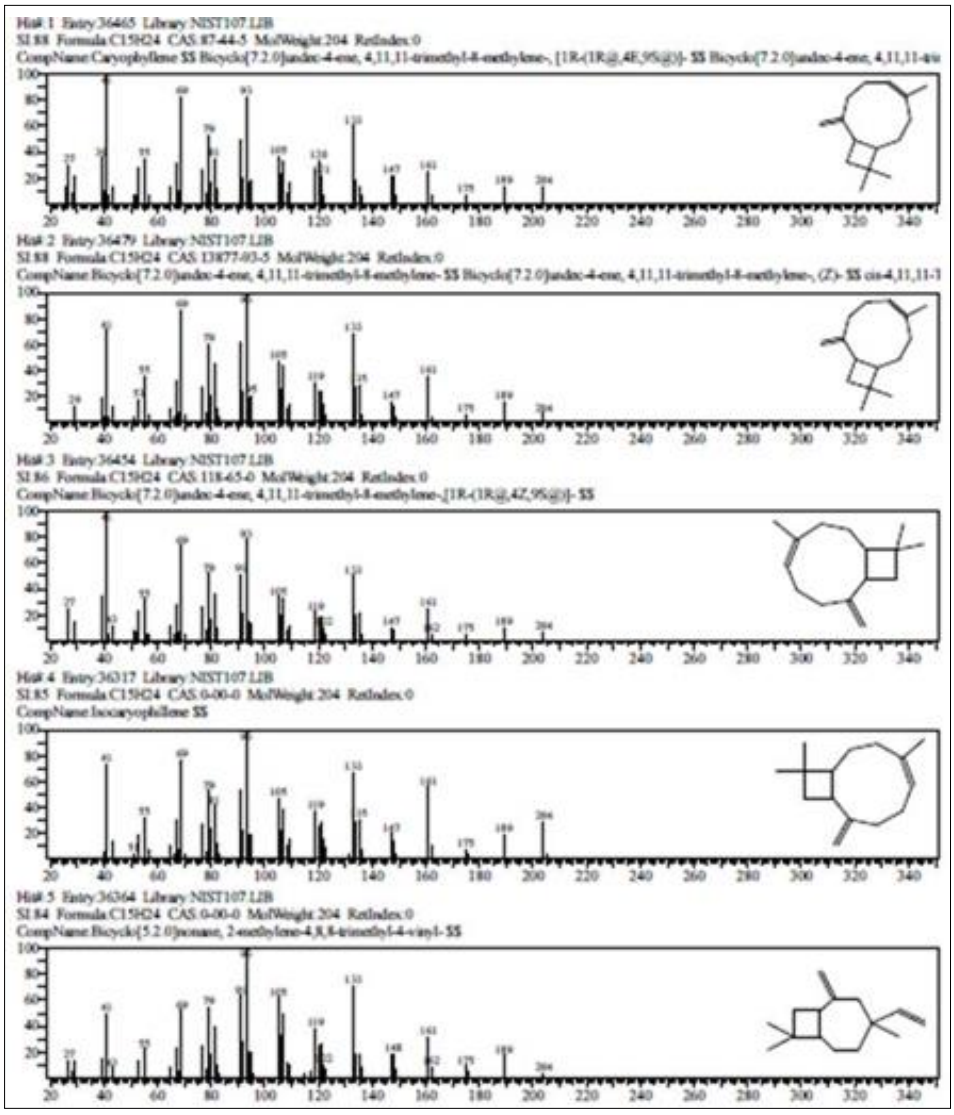

Fig 3: The GC-MS chromogram of methanolic extracts of *C. amada* rhizomes with peaks depicting Curcumene (b, g)

Chemical Composition of Extract

The results of GC/MS analysis of rhizome extracts of *C. amada* was presented as Figure 1 highlighting the most important and abundant secondary metabolite compounds such as curcumene (b,g). The identification of these compounds was carried out using the NIST library following to the standard methods.

Antibacterial activity

The zone of inhibition revealed that the *C. amada* rhizome extract (500 µg/disc) was efficient against different plant pathogenic fungi (Table 1). In comparison to the reference standard kanamycin (30µg/disc), maximum inhibition was obtained against Xanthomonas oryzae (15 mm) and Xanthomonas campestris (13 mm) (Table 1). The activity of *C. amada*'s rhizome extracts against Xanthomonas oryzae was demonstrated by their EC50 value of 173.24 µg mL−1. Its EC50 value of 483.17 µg mL−1 indicated that it had considerable activity against Xanthomonas campestris. When compared to Xanthomonas oryzae (<40 µg mL−1) and Xanthomonas campestris (<59 µg mL−1), carbendazim (30 µg/disc) shown a substantial difference.

Table 1: Antibacterial activities of the *C. amada* rhizome extract

Test bacterial strains	Diameter of Zone of Inhibition(mm)		EC_{50}	
	Extract 500 µg/disc	Std. Kanamycin 30 µg/disc	Extract 500 µg/disc	Std. Kanamycin 30 µg/disc
Xanthomonas oryzae	15	25	173.24	<40
Xanthomonas campestris	13	29	483.17	<59

Antifungal activity

Data on the fungicidal activity of synthetic fungicide carbendazim, which served as positive controls, and *C. amada* rhizome extracts are shown in Table 2. As compared to the reference standard carbendazim (30µg/disc), our results showed a high level of inhibition for *C. amada* rhizome extract (500 µg/disc) against Rhizoctonia solani (45 mm) and Magnaporthe oryzae (18 mm) (Table 2). The rhizome extracts of *C. amada* exhibited efficacy against R. solani, as evidenced by their EC50 value of 212.37 µg mL−1. Its EC50 value of 561.58 µg mL−1 indicated that it has moderate activity against Magnaporthe oryzae. Carbendazim demonstrated remarkable efficacy against two fungi: Magnaporthe oryzae (< 48 µg mL−1) and R. solani (< 28 µg mL−1).

Table 2: Antifungal activities of the *C. amada* rhizome extract

Test fungal strains	Diameter of Zone of Inhibition(mm)		EC_{50}	
	Extract 500 µg/disc	Std. Carbendezim 50 µg/disc	Extract 500 µg/disc	Std. Carbendezim 50 µg/disc
Rhizoctonia solani	45	50	212.37	<28
Magnaporthe oryzae	18	45	561.58	<48

Insecticidal activity

The data reported in Table 3 illustrate the *C. amada* rhizome extracts' pesticidal effectiveness against Nilaparvata lugens, a brown plant hopper. The findings demonstrated that 67.0% mortality was noted over a 72-hour exposure period at a 500 µg mL−1 concentration.

Table 3: Insecticidal activities of the *C. amada* rhizome extract

Extract	Concentration (mg/ml)	Insects (no's)	Number of insect dead				Mortality (%)
			0 h	24 h	48 h	72 h	
C. amada rhizome extract	500	15	0	4	7	10	67

Discussion

In the current study, we investigated the phenolic content, antioxidant, antibacterial, antifungal, and insecticide properties of *C. amada*. The maximum yield of tannins, flavonoids, and total polyphenols was obtained from methanol extracts. The polarity of the solvent used determined the amount of phenol present. In the past, methanol, ethanol, acetone, propanol, ethyl acetate, and dimethyl formamide have all been used to extract phenols (Tan *et al* 2012) [29]. The recovery of polyphenols from plant materials is greatly impacted by the degree of polymerization of the phenols, their solubility in the extraction solvent, the kind of solvent, the interaction of the phenols with other plant elements, and the formation of insoluble complexes (Galvez *et al* 2005) [11].

Alteration in the polarity of the solvents employed can be the cause of differences in the antioxidant activity. Furthermore, solvent polarity increases the solubility of phenols (Naczk and Shahidi, 2006) [17]. Owing to this, it can be challenging to establish a standardized procedure for the extraction of plant phenols. In general, the least polar solvents are considered suitable for extracting lipophilic phenols, while polar solvents are used for hydrophilic phenols, unless very high pressure is applied (Alothman *et al* 2009) [3].

It is complicated to ascertain the specific antioxidant capacity of each molecule because the plants contain a wide variety of antioxidants. Most methods developed to evaluate the antioxidant capacity of different plant materials (Swapana *et al*., 2013) [27] evaluate a material's ability to scavenge specific radicals by inhibiting lipid peroxidation or chelating metal. Our investigation into *C. amada* extracts revealed high total antioxidant and DPPH activity, as well as moderate ferrous iron reducing and carotene bleaching inhibitory capabilities. Mansouri *et al*. (2005) [16] suggested an interaction between antioxidant capacity and phenol content, emphasizing that phenols constitute the majority of plant antioxidant activity. From a structural perspective, phenols consist of an aromatic ring that has one or more hydroxyl substituents. The type of molecule's antioxidant activity is explained by its capacity to scavenge free radicals, donate hydrogen atoms or electrons, and bind metal cations (Amarowicz *et al* 2004) [4].

Plants are generally vulnerable to insect, microbial, and other attacks, and mammals usually produce a variety of secondary metabolites, such as steroids, alkaloids, terpenoids, and aromatic compounds, as a kind of self-defense that is repulsive or even lethal to the enemy (Fisher *et al*. 1984, Gurney and Mantle 1993) [10, 12]. In their study of plants used for insect control, Yang and Tang (1998) [30] found a strong correlation between pesticidal and medicinal plants. 10% of the world's food production is thought to be

lost each year to food grain storage insects, however losses of up to 25% can also occur in tropical countries as a result of insect assaults after harvest (Howe 1965) [14].

It was widely understood that prior phytochemical studies on the *C. amada* plant had identified flavonoids, steroids, triterpenoids, and alkaloids. The phyto-constituents of this type probably offer the *C. amada* rhizome extract its insecticidal properties. Drug resistance in phytopathogens can greatly decrease some pesticides' effectiveness (Rosenberger and Meyer 1981) [23].

Conclusion

This study highlights the potential use of medicinal plants, particularly *C. amada*, which has chemical components that may be employed as safe antibacterial agents and biopesticides. These substances are readily available, renewable, non-petrochemical, and naturally environmentally friendly.

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