

Phytochemical Profiling and Antifungal Activity of Essential Oil and Rhizome Extracts of *Curcuma Amada* Roxb



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Abstract

Curcuma amada (Zingiberaceae), commonly used as spice, and is also associated with multiple health benefits. In the present study, petroleum ether, cyclohexane, ethyl acetate, chloroform, acetone and methanol extracts of dried rhizome of *C. amada* prepared by solvent extraction were analyzed for quantitative and qualitative phytochemical profile and antifungal assay. *In vitro* antifungal activity of all extracts was determined by using agar well diffusion method against four fungi (*Rhizoctonia solani*, *Sclerotium rolfsii*, *Collectotricum falcatum* and *Sclerotinia solani*). The essential oil and extracts were found to active against the tested fungi in dose dependent manner by inhibiting the fungul mycelia growth. The essential oil of *C. amada* exhibited higher activity in comparison to extracts against all the fungi. Maximum inhibition of fungul growth was recorded for *R. solani* (80.93%) and *S. solani* (80.90%).

Keywords: *Curcuma amada*; Extracts; Essential oil; Phytochemicals; Antifungal activity

Research Article

The genus *Curcuma* belonging to the family Zingiberaceae has a widespread occurrence in the tropical Asia and Australia. This genus, comprises of more than 80 species of rhizomatous herbs including *Curcuma amada*, *Curcuma longa*, *Curcuma zedoaria*, *Curcuma aromatica*, is widely used in traditional systems of medicines such as Ayurveda, Siddha, Unani, Homeopathy and Naturopathy. In India, it is cultivated in innumerable agro-ecological situations right from the coastal areas to elevations as high as 1880m in the tropics and the sub-tropics of the country [1-3]. *Curcuma amada* Roxb. It is commonly known as Amada or 'Amahaldi' or 'mango ginger' due to the raw mango-like aroma of the rhizome. *Curcuma amada* Roxb.

Is a rhizomatous aromatic herb with a leafy tuft and 60-90cm in height? Leaves are long, petiolate, oblonganceolate, tapering at both ends, glabrous and green on both sides. Flowers are white or pale yellow, arranged in spikes in the centre of tuft of the leaves. Lip is semi-elliptic, yellow, 3-lobbed with the mid lobe emarginated [4]. *Curcuma amada* possess antifungal,

antiinflammatory, anticancer and anti hyperglyceridemic properties [5-7]. Rhizomes of *Curcuma amada* Roxb. Used for the manufacture of oleoresin and essential oil [8]. Its rhizomes essential oil containing β -myrcene, β -pinene, α -pinene, ocimene, ar-curcumene, linalool, linalyl acetate, camphor and safrole [9,10]. Based on the above facts it is imperative to investigate the qualitative phytochemical variations and antifungal activity in various extracts and essential oil.

Material and Methods

Plant material

Air dried rhizomes of *C. amada* were collected from the local agricultural field of District Udham singh nagar Uttarakhand, India and identified by Dr. D.S. Rawat (plant taxonomist), G.B.Pant University of Agriculture and Technology, Pantnagar. Rhizomes were washed thoroughly to remove adhering material and shade dried at room temperature and was further ground by means of an electrical blender to fine powder.

Isolation of the essential oil

The fresh rhizomes were hydro distilled for using a Clevenger type apparatus for 8h. The oil was extracted with the help of dichloromethane followed by drying over anhydrous Na₂SO₄. The yield of oil was found about 0.52% (w/v).

Preparation of plant extracts

Plant extracts were prepared in six different organic solvents (Petroleum ether, cyclohexane, ethyl acetate, chloroform, acetone and methanol) using solvent extraction [11]. Rhizome powder (300 g) was extracted in 150 ml of each solvent separately using Soxhlet extractor over water bath for 8h. The extracts were concentrated using vacuum rotatory evaporator at 45±5°C.

Phytochemical screening

Qualitative and quantitative analysis bioassay for total phenol [12], flavanols and orthodihydroxy phenols [13], phytochemical screening of all the six extracts for presence of alkaloids, tannin, anthraquinone, glycosides, reducing sugar, saponins, flavonoids, terpenoids, caumarine, emodins, anthocyanin, betacyanin was carried out according to standard method reported earlier [14-16].

Antifungal activity

The antifungal activity of different solvent extracts was determined by agar well diffusion method [17,18]. Four phytopathogenic fungi, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Colletotricum falcatum* and *Sclerotinia solani* were maintained and grown on potato dextrose agar medium in order to study antifungal activity of essential oil and various extracts having different polarity.

Sterilized petri plates of 90 mm diameter were used for pouring of medium. In each petri plate about 20 ml sterilized melted medium was aseptically poured near burner flame in a sterilized laminar air flow chamber. The medium in the plates were centrally inoculated by placing a 5 mm mycelial disc which was cut from the margin of 5 days old culture of the test fungus. Sterilized four filter paper disc were placed in a sterilized petri plates and different concentration of the extracts were added with the help of sterilized micropipette on each filter paper disc. The plates were sealed with parafilm immediately. Inoculated petri plates were incubated at 26±1°C in a BOD incubator. The growth of the fungus was measured in mm at an interval of 24 hours (18). Percent inhibition of growth was calculated by using the following formula:

$$I = \frac{C - T * 100}{C}$$

Where, I = Inhibition percentage, C = Colony radius in check (mm) T = colony radius in treatments (mm)

Results and Discussion

In present study, seeds of *C. amada* were extracted with six solvents with different polarity (petroleum ether, cyclohexane, ethyl acetate, chloroform, acetone and methanol) using Soxhlet apparatus. The yield of extracts mentioned in (Table 1). All the extracts were screened quantitatively in terms of their total phenols, flavonols and orthodihydroxy phenolic content with the help of their respective calibration curves (Figure 1). CAPE contained 91mg/100mg total phenols more than CAAC, CAEA, CAME, CACCL, CACH. CAME contained 26.31mg/100mg ortho dihydric phenolic contents more than CAPE, CAAC, CACL, CACH, CAEA. The flavonols content was observed more in CACH (75mg/100mg). The values are represented in catechol equivalent. The differences in the antioxidant activity of different extracts of *C. amada* may be possibly due to the different biochemical make up of the extracts in terms of phenols, flavonols and orthodihydroxy compounds and their concentration in the extracts.

Table 1: Extraction yields rhizome from *C. amada* (w/w of plant material).

Extracts	Amount of extract obtained	
	gm	%
Prtroleum eather extract(CAPE)	3.400	1.13
Cyclohexane extract(CACH)	0.810	0.27
Ethyl acetate extract(CAEA)	2.170	0.72
Chloroform extract(CACL)	2.326	0.77
Acetone extract(CAAC)	1.200	0.40
Methanol extract(CAME)	2.500	0.83

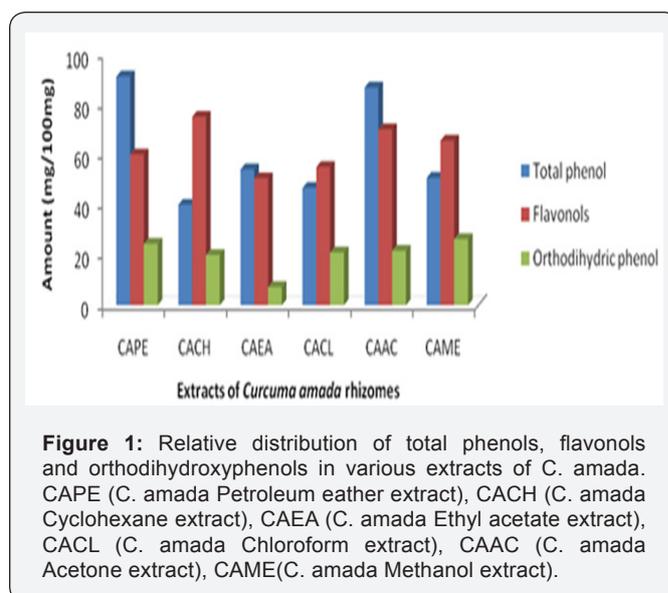


Figure 1: Relative distribution of total phenols, flavonols and orthodihydroxyphenols in various extracts of *C. amada*. CAPE (C. amada Petroleum ether extract), CACH (C. amada Cyclohexane extract), CAEA (C. amada Ethyl acetate extract), CACL (C. amada Chloroform extract), CAAC (C. amada Acetone extract), CAME(C. amada Methanol extract).

The phytochemicals such as phenolics, flavanoid have been reported to reduce the oxidative peroxidation of lipids by possessing antioxidant activity [19-21]. The chemical

composition and *in vitro* antioxidant potential of essential oil and rhizome extracts of *Curcuma amada* Roxb was reported in which essential oil was found to possess β myrcene over 40% as a major constituent (10). It has been reported by many workers all over the world that there exist a direct correlation among phenolic contents and antioxidant activity [22,23] (Figure 1).

For qualitative secondary metabolite profiling twelve phytochemicals viz. alkaloids, tannin, anthraquinone, glycosides,

reducing sugar, saponins, flavonoids, terpenoids, caumarine, emodins, anthocyanin, and betacyanin were analyzed. The study revealed that alkaloids was present only in CAME. Tannin and saponins could not be detected in any extracts. Anthraquinone could be detected only present in CAEA. Glycosides and emodins were present in CAME. Betacyanin, caumarine and reducing sugars were present in CAEA. Flavonoids, terpenoids were present in all extract. Anthocyanin was present in CAPE, CACH, CAAC and CAME (Table 2).

Table 2: Phytochemicals detected in different extracts from *C. amada* rhizomes.

Phytochemicals	CAPE	CACH	CAEA	CACL	CAAC	CAME
Alkaloids	-	-	-	-	-	+
Tannin	-	-	-	-	-	-
Anthraquinone	-	-	+	-	-	-
Glycosides	-	-	-	-	-	+
Reducing sugar	-	-	+	-	-	-
Saponins	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Caumarine	-	-	+	-	-	-
Emodins	-	-	-	-	-	+
Anthocyanin	+	+	-	-	+	+
Betacyanin	-	-	+	-	-	-

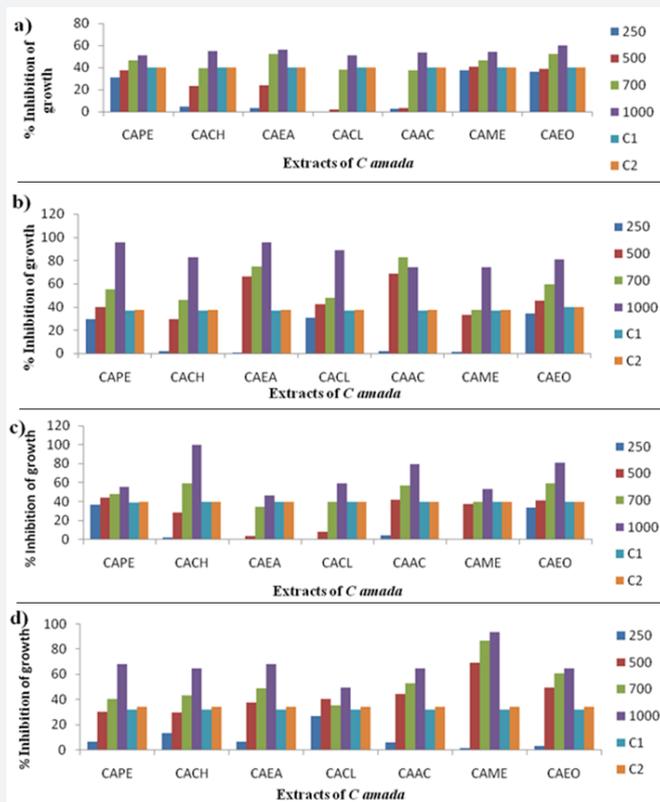


Figure 2: Percent inhibition of fungal mycelia growth of a) *C. falcatum*, b) *S. solani*, c) *R. solani*, d) *S. rolfsii* against various extracts, CAPE (*C. amada* Petroleum ether extract), CACH (*C. amada* Cyclohexane extract), CAEA (*C. amada* Ethyl acetate extract), CACL (*C. amada* Chloroform extract), CAAC (*C. amada* Acetone extract), CAME (*C. amada* Methanol extract) and CAEO (*C. amada* essential oil). C-1 indicates control (acetone) & C-2 indicates blank (without acetone).

The rhizome essential oil and all the extracts were found active against the tested fungi in dose dependent manner by inhibiting the fungal mycelia growth. All the extracts exhibited good to moderate activity against *C. falcatum* but the maximum inhibition dose recorded for CAEA (55.90%) at 1000ppm of dose level at 72 hrs. The other extracts inhibited 54.15-50.95% of mycelia growth in order of CACH (54.75%) >CAME (54.15%) >CAAC (53.38%) >CACL (51.05%) >CAPE (50.95%) respectively at the same dose level (Figure 2). Against *S. solani* all the extracts exhibited well to moderate activity but the maximum inhibition dose recorded for CAPE and CAEA (95.57%) at 1000ppm of dose level at 72 hrs. The other extracts inhibited 88.59 -73.89% of mycelia growth in order of CACL (88.59%) > CACH (82.86%) > CAME (73.95%) >CAAC (73.89%) respectively at 1000ppm of dose level at 72 hrs (Figure 2). CACH was found to be most effective against *R. solani*, it exhibited 100% growth at 1000ppm of dose level at 72 hrs.

The other extracts inhibited 79.78% - 46.70% of mycelia growth in order of CAAC (79.78%) > CACL (59.18%) > CAPE (55.66%) > CAME (53.40%) >CAEA (46.70%) respectively at 1000ppm of dose level at 72 hrs (Figure 2). Against *S. rolfsii* the extracts exhibited good antifungal activity but the maximum inhibition dose was recorded for CAME (93.25%) at 1000ppm of dose level at 96 hrs. The other extracts inhibited 68.00 - 49.34% of mycelia growth in order of CAPE (68.00%) > CAME (64.45%) > CACH & CACL (64.43%) >CAEA (49.34%) respectively at 1000ppm of dose level at 96 hrs As per the study the essential oil and all the extracts were found to active against the tested fungi (*Rhizoctonia solani*, *Sclerotium rolfsii*, *Collectotricum falcatum* and *Sclerotinia solani*) in dose dependent manner by inhibiting the fungal mycelia growth.

The essential oil of *C. amada* exhibited higher activity in comparison to extracts against all the fungi. The volatile oil from mango ginger rhizomes has antifungal in nature and it has been reported that myrcene and pinene possess antifungal activity against the wide range of fungi, viz. *Curvularia palliscens*, *Aspergillus niger*, *A. terreus*, *Fusarium moniliforme* and *F. falcatum* [24]. The major constituents of the essential oil have been reported by our group (10) also possess β -myrcene and β -pinene as the major constituent. Thus the present result of essential oil for possessing antifungal activity against tested pathogenic fungi was justified by the results reported earlier (Figure 2).

Conclusion

With the increased resistance towards synthetic drugs in phytopathogenic fungi, plant products may provide a better alternative to cure as well as prevent the infections caused by them. The present study revealed significant antifungal potential of *Curcuma amada* along with providing an easy, economic and less polluting way to extract out target bioactive molecules. However further investigations regarding the isolation of individual component from most active extracts may help in

offering the natural alternative to treat infections caused by investigated fungus.

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