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Research Article

## Phytochemical Analysis and Antioxidant Potential of Rhizome Extracts of *Curcuma amada* Roxb and *Curcuma caesia* Roxb

Mamta Yadav\*, K. Kalyan Saravanan

Bhagwant University, Sikar Rd, Ajmer, Rajasthan 305004

### ABSTRACT

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. *Curcuma amada* Roxb (*C. amada*, Zingiberaceae) is a perennial, rhizomatous, aromatic herb commonly known as Amada or Amahaldi or mango ginger due to the raw mango-like aroma of the rhizome. It is used medicinally as a coolant, astringent and to promote digestion. *Curcuma caesia* Roxb (*C. caesia*, Zingiberaceae) is a perennial herb with bluish black rhizomes commonly known as black turmeric and are traditionally used in treatment of various ailments and metabolic disorders like leukoderma, asthma, tumours, piles, bronchitis, etc. in Indian system of medicine. The aim of the present study was to evaluate *in vitro* antioxidant activities and qualitative and quantitative phytochemical analysis of rhizome of *C. amada* and *C. caesia* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of methanolic extract of the rhizome was assessed against DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total flavonoids content of methanolic rhizomes extract of *C. caesia* and *C. amada* was found to be 2.752 and 2.920 mg/100mg respectively. The activities of methanolic rhizomes extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 14.11 and 170.81, 63.69µg/ml 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*, *Curcuma caesia*, Zingiberaceae, Phytochemical analysis, Antioxidant

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### \*Address for Correspondence:

Mamta Yadav, Bhagwant University, Sikar Rd, Ajmer, Rajasthan 305004

### INTRODUCTION

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments<sup>1</sup>. The genus *Curcuma* is a well known spice of India. It is also called Haldi and more than 200 species and subspecies of it is found all across the world. One of which is *Curcuma caesia* Family: Zingiberaceae. It is also known as Kali Haldi. It is an erect rhizomatous herb with large leaves. Fresh rhizomes are aromatic with intense camphoraceous odour and are applied externally to sprain and bruises<sup>2</sup>. *C. caesia* is native to Northeast and Central India. It is also sparsely found in Papi hills of East Godavari, the root hills of the Himalayas and North Hill forest of Sikkim. The rhizomes of *C. caesia* have a high economic importance owing to its putative medicinal properties. The rhizomes are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy,

fever, wound, vomiting, menstrual disorder, smooth muscle relaxant activity, anthelmintic, aphrodisiac, inflammation, gonorrhoeal discharges, etc<sup>3, 4</sup>. *C. Caesia* contain maximum curcuminoids, oil content, flavonoids, phenolics, different important amino acids, protein and high alkaloid content which reveals that the presence of these bioactive secondary metabolites correlates with the medicinal uses of *C. Caesia* as fragrances, flavouring and many important useful pharmaceutical products<sup>5</sup>. *C. amada* Roxb (Zingiberaceae) is a perennial, rhizomatous, aromatic herb commonly known as Amada or Amahaldi or mango ginger due to the raw mango-like aroma of the rhizome. It is found wild as well as in cultivation in various parts of world. In India, it is cultivated in Gujarat, West Bengal, Uttar Pradesh, Karnataka, Tamil Nadu, Konkan and in the hills of Western coast of India but it is not cultivated anywhere commercially<sup>6</sup>. Mango ginger is used medicinally as a coolant, astringent and to promote

digestion. In addition, to this, it is used as a basic ingredient in pickles, preserves, candies, sauces, curries and salads<sup>7,8</sup>. Its rhizome has carminative properties as well as being useful as a stomachic<sup>9</sup>. Its rhizome has traditionally been used for healing of wounds, cuts and itching<sup>10</sup>. It possesses antifungal, anti-inflammatory, analgesic, anticancer and antihyperglyceridemic properties<sup>11</sup>. The rhizome extracts of *Curcuma* were observed to be potent antimutagenic properties based on its antioxidative activity<sup>12</sup>. Its rhizomes are used for the manufacture of oleoresin, essential oil, etc<sup>13</sup>. It reported to contain ocimene, dihydro-ocimene,  $\alpha$ -pinene,  $\alpha$ -curcumene,  $\beta$ -curcumene, linalool, cuminyl alcohol, keto-alcohol, camphor, turmerone, linalyl acetate, safrole, curcumin, myristic acid, car-3-ene, myrcene, 1,8-cineol, limonene and perillene<sup>14</sup>. The rhizomes are used by the tribals of Madhya Pradesh for the management of diabetes mellitus<sup>15</sup>. Antioxidants present in vegetables, beverages and fruits were providing health-promoting ingredients in human diet and also responsible for the prevention and treatment of radical-mediated disorders<sup>16</sup>. The usefulness of artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are under scrutiny due their suspected role in carcinogenesis<sup>17</sup>. Thus, there is an urgent need of natural additives as potential antioxidants having an important role in preventing a variety of stress-related diseases<sup>18</sup>. The present study was focused to evaluate the phytochemical analysis and anti oxidant activity of rhizomes of *C. amada* and *C. caesia*.

## MATERIALS AND METHODS

### Plant material

Rhizome of *C. Caesia* and *C. Amada* were collected from local area of Bhopal (M.P.) in the month of March, 2019.

### Defatting of plant material

Powdered plant material of *C. Caesia* and *C. Amada* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

### Extraction by maceration process

265gm of *C. Caesia* and 310 gm *C. Amada* dried Rhizome were exhaustively extracted with different solvent (chloroform, ethyl acetate, methanol and water) using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts<sup>19,20</sup>.

### Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

### Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures<sup>21,22</sup>. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

### Total flavonoid contents

The total flavonoid content was determined using the method of Olufunmiso et al<sup>23</sup>. 1 ml of 2% AlCl<sub>3</sub> methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

### Antioxidant activity of extract using DPPH method

DPPH scavenging activity was measured by modified method<sup>23</sup>. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100  $\mu$ g/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control]  $\times$  100%. Though the activity is expressed as 50% inhibitory concentration (IC<sub>50</sub>), IC<sub>50</sub> was calculated based on the percentage of DPPH radicals scavenged. The lower the IC<sub>50</sub> value, the higher is the antioxidant activity.

## RESULTS AND DISCUSSION

The crude extracts so obtained after each of the maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the rhizomes of the plants using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder rhizomes of *C. caesia* and *C. amada* are shown in Table 2 & 3. methanolic and aqueous extracts of rhizomes sample of *C. caesia* and *C. amada* showed the presence of flavonoids, phenols, tannins, carbohydrate, glycosides and proteins but in chloroform extracts all phytoconstituents was absents and flavonoids was present in *C. amada* extract.

Table 1 Results of percentage yield of rhizomes extracts

Plant Name	Percentage yield (%)				
	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Water
<i>C. caesia</i>	0.780	1.91	0.426	1.83	3.13
<i>C. amada</i>	0.875	2.90	1.04	2.66	3.15

Table 2 Phytochemical evaluation of *C. caesia* rhizomes extracts

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	-	-	-	-
2.	Glycosides	-	-	-	-
3.	Flavonoids	-	-	+	+
4.	Saponins	-	-	+	+
5.	Phenolics	-	-	-	-
6.	Amino Acids	-	-	-	-
7.	Carbohydrate	+	+	+	+
8.	Proteins	-	-	+	+
9.	Diterpenes	+	+	+	+

(+) Indicates 'Presence'; (-) Indicates 'Absence'

Table 3 Phytochemical evaluation of *C. amada* rhizomes extracts

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	-	-	-	-
2.	Glycosides	-	-	-	-
3.	Flavonoids	-	+	+	+
4.	Saponins	-	-	+	+
5.	Phenolics	-	-	-	-
6.	Amino Acids	-	-	-	-
7.	Carbohydrate	+	+	-	-
8.	Proteins	+	+	+	+
9.	Diterpenes	-	+	+	+

(+) Indicates 'Presence'; (-) Indicates 'Absence'

The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of methanolic, aqueous and ethyl acetate extracts of rhizomes of *C. amada* showed the content values of 2.920, 2.717 and 2.895 respectively and TFC of methanolic and aqueous extract of *C. caesia* was 2.752 and 2.440 respectively Table 4 & Fig.1. Antioxidant activity of the samples was calculated through

DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 5 & Fig 2. In comparison to both the plant *C. amada* have higher antioxidant activity as comparison to *C. caesia*.

Table 4 Estimation of total flavonoids content of *C. caesia* and *C. amada*

S. No	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)
<b><i>C. caesia</i> extracts</b>		
1	Methanol	2.752
2	Aqueous	2.440
<b><i>C. amada</i> extracts</b>		
1	Ethyl acetate	2.895
2	Methanol	2.920
3	Aqueous	2.717

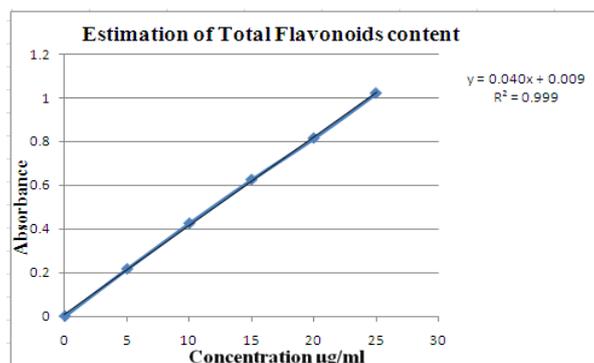
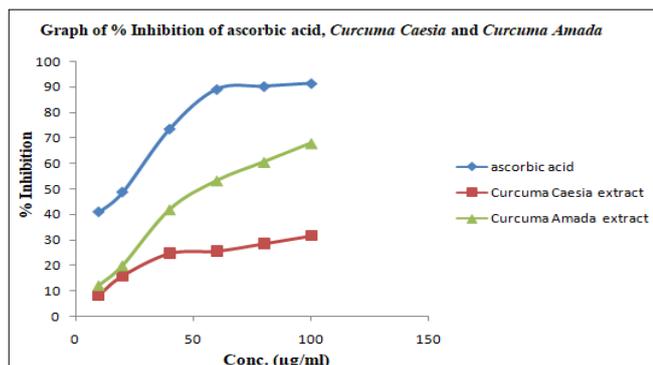


Figure 1 Graph of estimation of total flavonoids content

Table 5 % Inhibition of ascorbic acid, *C. caesia* and *C. amada* methanolic extract using DPPH method

S. No.	Conc. (µg/ml)	Ascorbic acid % Inhibition	<i>C. caesia</i> % Inhibition	<i>C. amada</i> % Inhibition
1	10	40.92	8.26	11.94
2	20	48.70	15.77	19.75
3	40	73.48	24.65	41.8
4	60	89.04	25.57	53.13
5	80	90.20	28.48	60.49
6	100	91.35	31.54	67.84
	IC 50	14.114	170.81	63.69

Figure 2 Graph of % Inhibition of ascorbic acid, *C. caesia* and *C. amada* using DPPH method

## CONCLUSION

It can be concluded that from present investigation The phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The potential antioxidant activity of organic extracts of *C. amada* and *C. caesia* rhizomes indicate its protective role against oxidative damage and as an important natural antioxidant. The curcumin of curcuminoid family found in spice turmeric is a potent antioxidant scavenging ROS and induced antioxidant response<sup>24</sup>. The extract due to its antioxidant activity could be utilized in pharmaceutical sector and food industries. Further research in this direction will be utilized for strengthening its real potential in various sectors.

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