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Phytochemical Comparison and Quantification in Curcuma longa L. and Curcuma amada Roxb

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Abstract:

Curcuma longa L. (turmeric) and Curcuma amada Roxb. (Mango ginger) are two economically and medicinally important species of the Zingiberaceae family, widely used in traditional Indian medicine and culinary practices. This study aimed to conduct a comparative phytochemical analysis of these two species, focusing on their total phenol and flavonoid content. Preparation and determination of extractive values of rhizomes from both species using various solvents evaluated. Phytochemical scanning showed the presence of alkaloids, tannins, saponins, terpenoids, and glycosides in both species, but to different degrees. Both the species of Curcuma showed significant phenol and flavonoid content.

Keywords: Extractive value, phytochemical analysis, C. longa L., C. amada Roxb.

Introduction:

Since time immemorial Turmeric, a member of the Zingiberaceae family, is a widely studied medicinal plant due to its extensive phytochemical composition and therapeutic properties. Among the various species of turmeric, *Curcuma longa* L. and *Curcuma amada* Roxb. are particularly significant in traditional Indian medicine and culinary practices. *Curcuma longa* L., commonly known as turmeric, is renowned for its active compound curcumin, which exhibits anti-inflammatory, antioxidant, and anticancer properties (Prasad & Agarwal, 2011). On the other hand, *Curcuma amada* Roxb., known as mango ginger, is valued for its unique mango - like aroma and similar bioactive properties, though it is less extensively studied compared to *C. longa* L. (Ravindran et al., 2007).

According to Jatoi, S. A., *et al.* (2007), these spices offer unique health advantages when used together. They can reduce fever and act as an aphrodisiac. They can also help with urine flow, soften skin, and clear mucus, also they can ease constipation. These spices are also known to help with liver issues and skin problems. They may also relieve asthma, bronchitis, hiccups, fever, itching, and swelling.

Phytochemicals such as phenols and flavonoids are crucial bioactive compounds that contribute to the antioxidant potential of these plants. Phenolic compounds are known for their ability to scavenge free radicals, thereby reducing oxidative stress, while flavonoids play a significant role in modulating cellular signalling pathways and enhancing immune responses (Pandey & Rizvi, 2009). Comparative studies on the phytochemical composition, total phenol, and flavonoid content of *C. longa* L. and *C. amada* Roxb. are essential to understanding their relative medicinal efficacy and potential applications in nutraceuticals and pharmaceuticals (Tariq A. L., 2016).

In India, where both species are cultivated and utilized extensively, there is a

growing interest in exploring their phytochemical profiles to validate their traditional uses and promote their commercial exploitation. However, limited comparative studies have been conducted on these two species, particularly in the Indian context.

This study aims to provide a detailed comparative analysis of the phytochemical constituents, total phenol, and flavonoid content in *C. longa* L. and *C. amada* Roxb. with a focus on Indian varieties. Such research will not only enhance our understanding of their bioactive potential but also contribute to the development of value added products from these economically important plants.

Materials and methods:

Collection of the Plant Material:

Rhizome part of *Curcuma longa* L. and *Curcuma amada* Roxb., was used for the study. The Crude fine powder of rhizome of both plants was directly purchased from the local market. The powder was again sieved to remove any debris and stored in an airtight container.

Preparation of plant Extract:

The powder was extracted with 100 ml of Methanol, Ethanol, Acetone, water, and Petroleum ether using the Soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure below 40ºC to dryness till the extract evaporated. Crude dried extracts were stored in the refrigerator at 4ºC for their future use in screening for phytochemical analysis.

Chemicals and Standards:

Gallic acid, Quercetin, Sodium carbonate, Folin – Ciocalteu reagent, Sodium nitrate, Aluminium chloride, Sodium hydroxide, Methanol, ethanol, Acetone, Petroleum ether and Distilled water.

Determination of Extractive value (Pawar S.S, *et al* ., 2015):

2 gm of finely powdered crude drug was placed in a conical flask and mixed with the solvents (30 ml) Methanol, ethanol, acetone, water, and Petroleum ether separately. The flasks were covered with foil and kept aside for 24 hrs at room temperature shaking frequently. After 24 hrs the mixtures were filtered through Whatman filter paper no. 1. The obtained filtrates of various solvents were then transferred to pre – weighted beaker, and reduced to dryness by keeping the filtrate for complete evaporation of solvents. The extractive value in percentage was calculated by using the following formula and recorded.

Extractive value (%) = Weight of dried extract / Weight of plant material *100.

Phytochemical Analysis (Banu, K. S, *et al* ., 2015, Shaikh, J. R., *et al.*, 2020)

The tests were carried out using Methanol, ethanol, acetone, water, and Petroleum ether extracts to identify the presence of various active constituents using the standard procedures.

1. Test for alkaloids: 1ml of Wagner's reagent was mixed with 1ml of extract, formation of a Reddish brown precipitate indicated the presence of Alkaloids.
2. Test for phenols: 1 ml of Extract was mixed with 1 ml of 5% solution of $FeCl_3$. A black coloration indicated the presence of phenols.
3. Test for flavonoids: 1ml of the extract was mixed with 1ml of 10 % NaOH solution (+ few drops dil. HCl), formation of an intense yellow colour ppt which disappears after the addition of HCl indicated the presence of flavonoid.
4. Test for tannin: 1 ml of Extract was dissolved in 5mL distilled water and 1ml of 1% gelatine solution and a pinch of NaCl. The formation of white ppt indicated the presence of tannins.
5. Test for terpenoids: (Salkowski's test) 1 ml of extract was treated with a few drops of conc. H_2SO4 (Shaken well and allowed to stand), Red colour (in lower

layer) indicated the presence of terpenoids.

6. Cardiac Glycosides (Keller-Kilani test): 1ml of extract mixed with 1.5mL glacial acetic acid a drop of 5% ferric chloride and conc. H_2SO_4 (along the side of the test tube) A blue coloured solution indicated the presence of Cardiac Glycosides.
7. Test for Steroids (Salkowski's test) – 1ml of extract mixed with a few drops of conc. H_2SO_4 (Shaken well and allowed to stand) Red colour (in lower layer) indicated the presence of Steroids.
8. Test for saponins – 1 ml of extract was shaken with 5 ml of distilled water; foam appears if persisted for 10 minutes indicating the presence of Saponins.
9. Test for Carbohydrate - Fehling's test 1mL of each of Fehling's solutions A & B mixed with 1ml of extract and boiled in the water bath, Brick red precipitate indicated the presence of reducing sugar.
10. Test for Protein: (Xanthoproteic test) – A few drops of Nitric acid mixed with 1ml of extract. The formation of an intense yellow colour indicated the presence of protein.

Standard solution preparation:

The standard solution was prepared by weighing 10 mg of gallic acid in 10ml of Methanol and 10 mg of quercetin and dissolving it in 10 ml of methanol, making the concentration of the solution [1 mg/ml].

Determination of total Phenolic content:

The total phenolic content in the extracts was measured using the Folin – Ciocalteu reagent by (Sahu, R., *et. al.,* 2013).Gallic acid was used as a standard and the total phenolics were expressed as mgGAE/g gallic acid To achieve this, a calibration curve for gallic acid was made. Standard solutions of 25, 50, 75, 100 and 125 µg/ml concentrations of gallic acid were prepared in methanol. A 1 mg/ml concentration of plant extract was also prepared in methanol, and 0.5 ml of each sample was added to test tubes, which were subsequently mixed with 2.5 ml of a 10-fold diluted Folin – Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. The test tubes were kept at room temperature for 90 minutes, and the absorbance was measured at 760 nm spectrometrically

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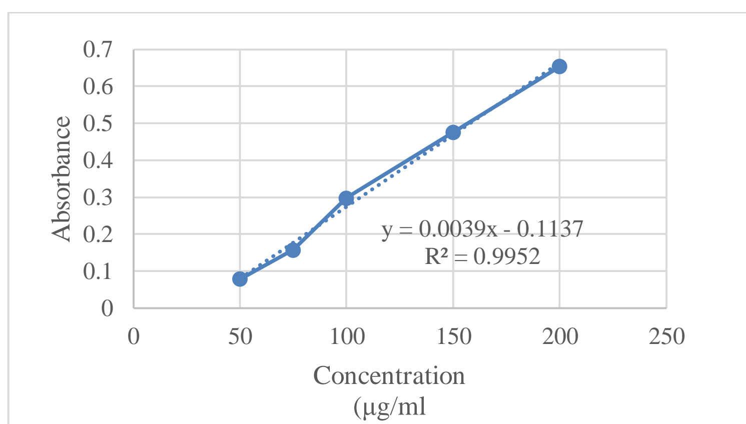
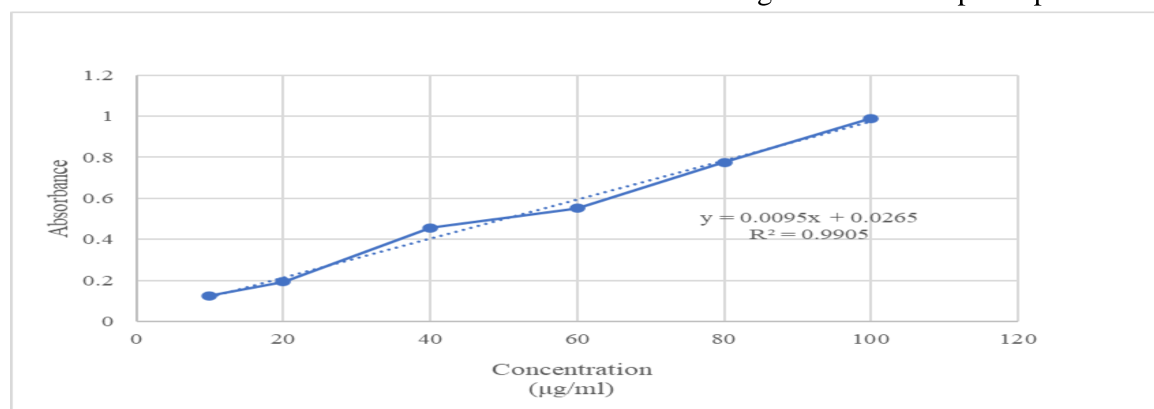


Total Flavonoid content (Sahu, R., *et al.,* 2013):

The aluminium chloride method was used for flavonoid determination. In this method, quercetin was used as standard, and flavonoid contents were measured as quercetin equivalent mgQE/g. For this purpose, the calibration curve of quercetin was drawn as shown in a standard graph curve. 1ml of standard solution (10, 20, 40, 60, 80,100 µg/ml) was taken into a 10 ml volumetric flask, containing 4ml of distilled

water. 0.3 ml of 5 % $NaNO_2$ added to the flask. After 5 min, 0.3 ml 10 % $AlCl_3$ was added to the mixture. At the 6th minute, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. A similar procedure was done with plant extract. The absorbance was noted at 510 nm using a UV-visible spectrophotometer.



S. No.	Solvent	Weight of the crude drug (g)	Extractive value (*Curcuma longa*) (%/w/w)	Extractive value (*Curcuma amada*) (%w/w)
1	Methanol	2	31.4	17.9
2	Ethanol	2	18.7	12.5
3	Acetone	2	9.3	8.14
4	Water	2	15.9	12.67
5	Petroleum ether	2	6.3	2.7

Table 1: Extractive value of *Curcuma longa L. and Curcuma amada Roxb.*

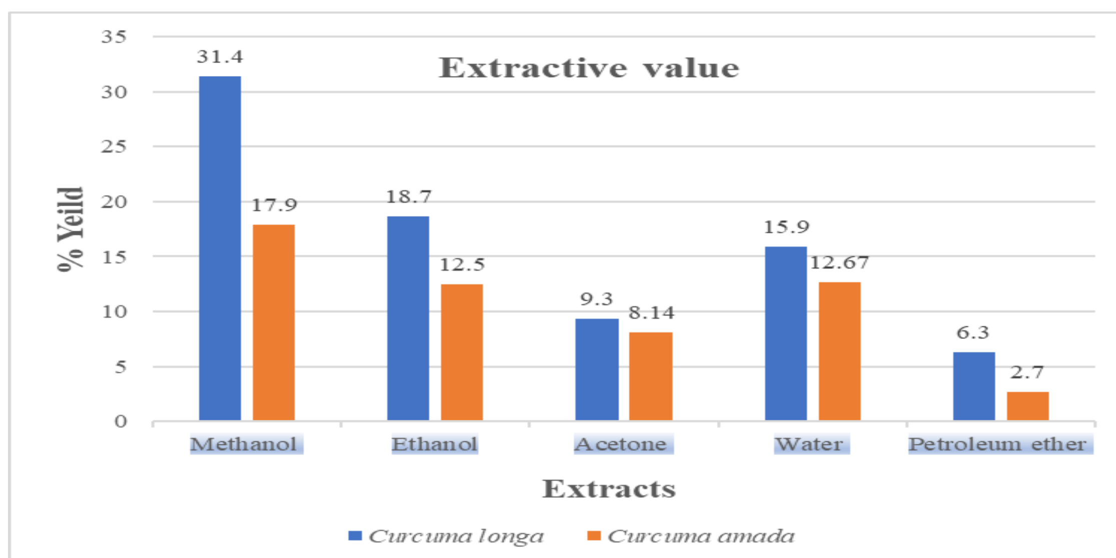


Figure No 1. Extractive values *Curcuma longa and Curcuma amada.*

Phytochemical Test Results for *Curcuma longa L.*

Phytochemical	Methanol Extract	Ethanol Extract	Acetone Extract	Aqueous Extract	Petroleum Ether Extract
Alkaloids	+	+	+	-	-
Flavonoids	+	+	+	+	-
Terpenoids	+	+	+	+	+

Saponins	-	-	-	+	-
Tannins	+	+	+	+	-
Phenolic Compounds	+	+	+	+	-
Glycosides	+	+	+	+	-
Steroids	+	+	+	-	+
Carbohydrates	-	-	-	-	-
Proteins	-	-	-	-	-

Table 2 . Phytochemical screening of *Curcuma longa L (+ signindicates presence , - sign indicates absence)*

Phytochemical Test Results for *Curcuma amada Roxb.*

Phytochemical	Methanol Extract	Ethanol Extract	Acetone Extract	Aqueous Extract	Petroleum Ether Extract
Alkaloids	+	+	+	-	-
Flavonoids	+	+	+	+	-
Terpenoids	+	+	+	+	+
Saponins	-	-	-	+	-
Tannins	+	+	+	+	-
Phenolic Compounds	-	-	+	+	-
Glycosides	+	+	+	+	-
Steroids	+	+	+	-	+
Carbohydrates	-	-	-	+	-
Proteins	-	-	-	-	-

Table 3. Phytochemical screening of *Curcuma amada Roxb. (+ sign indicates presence , - sign indicates absence)*

Extract	Total Phenolic content (mg GAE/g)	Total FlavonoidContent (mg QE/g)
C. longa	153.33 ± 10.21	38.89 ± 3.55
C.amada	72.18 ± 9.76	11.14035 ± 0.90

Table 4. Total phenolic and Flavonoid Content.

Result and Discussion:

The extractive capability of any solvent quantifies its effectiveness in isolating bioactive compounds from a specific sample.Solvents that exhibit a high extractive potential are anticipated to be proficient in drawing outbioactive components. There exists a notable difference at $P < 0.05$ in the extractive efficiencies of all solvents for turmeric and Mango ginger.

Polar solvents are believed to show elevated extractive capabilities as most active compounds tend to be polar (Arawande, J. O., *et al.*, 2018). The extractive value of *Curcuma longa* L. and *Curcuma amada* Roxb. in Methanol, ethanol, acetone, water, and Petroleum ether extract were determined in (Figure 1). Extractive value provides the yield percentage of phytoconstituents in the solvent. The result is shown in Table 1. The extractive value of Methanol and Ethanol extract of *Curcuma longa* L. was found to be maximum (31.4% and 18.9%). Water extract showed moderate extractive value (15.9%). The Acetone and petroleum ether extract showed slightly less extractive value 9.3%

and 6.35%. The extractive value of Methanol, Ethanol, and water extract of Curcuma *amada* Roxb. was found to be maximum (17.9%w/w, 12.5%, and 12.67w/w). The Acetone (8.14 %), and Petroleum ether (2.7%) extract of *Curcuma amada* Roxb. showed very less extractive value.

The phytochemical screening of *Curcuma longa* L. and *Curcuma amada* Roxb. extracts in different solvents revealed the presence and absence of various bioactive compounds as shown in Table 2 and 3. The findings indicate that different solvents extract different classes of phytochemicals based on their polarity. Alkaloids were detected in methanol, ethanol, and acetone extracts but were absent in aqueous and petroleum ether extracts. Flavonoids were present in all extracts except for petroleum ether extract. Terpenoids were consistently present in all extracts tested. Saponins were found only in the aqueous extract. Tannins and Phenolic Compounds were detected in methanol, ethanol, acetone, and aqueous extracts but were absent in petroleum ether extract. Glycosides were found in all extracts except petroleum ether. Steroids were present in methanol, ethanol, acetone, and petroleum ether extracts but absent in the aqueous extract. Carbohydrates and proteins were not detected in all extracts.

Plants contain various metabolites, including primary metabolites and secondary metabolites. Primary metabolites, such as proteins, lipids, carbohydrates, and amino acids, can be found in various parts of the plant; these metabolites are essential for the plant's growth and development. In contrast, secondary metabolites like tannins, saponins, flavonoids, phenolics, alkaloids, terpenoids, steroids, etc., are present in the plant parts to protect the plants from disease-causing germs/pathogens or under any unfavourable conditions. Also, the preliminary phytochemical analysis is not sufficient to evaluate the content of secondary metabolites present in the extract. Therefore, the quantitative phytochemical analysis was carried out in this study to evaluate the contents of phytochemicals including Phenolic and flavonoid in the rhizome sample of C. *longa* L. and *C. amada* Roxb. (Sutar J., *et al*., 2020). The present study revealed the phenol contents as mg equivalent mgGAE/g of dry sample at (standard plot: $y = 0.0039x - 0.1137$, $R^2 = 0.9952$).The quantitative analysis of the Total Phenolic Content (TPC) of methanolic extract of *Curcuma longa* L. contained the highest amount of TPC (153.33 ± 10.21mgGAE/g), followed by 72.18 ± 9.76 in *Curcuma amada* Roxb. Similarly Using the standard plot of quercetin ($y = 0.0095x$, $R2 = 0.9905$), the total flavonoid content of methanolic extract of *Curcuma longa* L. contained the highest, 38.89 ± 3.55 (mg QE/g) amount of TFC followed by 11.14035 ± 0.90 (mg QE/g)as Shown in table 4.

Conclusion:

The present study revealed that the *Curcuma longa* L. and *Curcuma amada* Roxb. contain significant amount of phenols and flavonoids. The results showed that both *C. longa* L. and *C. amada* Roxb. possess a rich array of phytochemicals, including curcuminoids, terpenes, and other bioactive compounds. Curcuminoids, particularly curcumin, were found to be the predominant compounds in *C. longa*, L. while *C. amada* Roxb. exhibited a more diverse profile of phytochemicals. (Katsuyama, *et al.,* 2007) The total phenolic content and total flavonoid content were significantly higher in *C. longa* L. compared to *C. amada* Roxb., suggesting that *C. longa* L. may have a greater potential for pharmacological and therapeutic applications. (Miyazaki, *et al.,* 2014). These findings contribute to the ongoing research on the medicinal properties of *Curcuma* species and provide valuable

insights into the phytochemical differences between *C. longa* L. and *C. amada* Roxb. (Itokawa, *et al*., 2008) (Katsuyama, *et al.,* 2007) (Singh, *et al.,* 2020) (Miyazaki, *et al.,* 2014).

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