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A Comparative Analysis of Phytochemical Content and Extractive Efficiency in Curcuma Species: Profiling Total Phenolics, Flavonoids, and Alkaloids

Mali Seeta Vitthal*[1], Vishal Gupta[2]

Faculty of Pharmacy, Mansarovar Global University-[MGU], Sehore (Madhya Pradesh)
Corresponding author mail id: aditi.zagadesvpm@gmail.com

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ABSTRACT

The present investigation explores the comparative phytochemical profiles, extractive efficiencies, and bioactive compound content of three Curcuma species, Curcuma zedoaria, Curcuma caesia, and Curcuma amada using solvents of varying polarities: ethyl acetate, ethanol, and water. Extraction yields varied among species, with aqueous extracts showing the highest yields in C. caesia (1.94%) and C. amada (2.28%), while ethanol proved most effective for C. zedoaria (1.36%), indicating a solvent-dependent distribution of phytoconstituents. Phytochemical screening revealed distinct patterns of bioactive compounds across species and extracts. Ethanolic extracts of C. caesia and C. amada showed a rich diversity of secondary metabolites, including alkaloids, flavonoids, phenols, and diterpenes. Quantitative analysis supported these findings, with C. caesia exhibiting the highest phenolic (2.56 mg/100 mg GAE) and flavonoid (2.97 mg/100 mg QE) content, while C. amada had the highest alkaloid content (1.43 mg/100 mg AE). C. zedoaria, although less chemically diverse, demonstrated notable extractive efficiency with ethanol and may possess selective antimicrobial potential. Overall, C. caesia emerged as the most phytochemically potent species, while C. amada showed promising alkaloid-associated bioactivity. These findings provide a scientific foundation for the selection of appropriate Curcuma species and extraction methods for therapeutic, nutraceutical, or pharmacological applications.

Keywords: Curcuma zedoaria, Curcuma caesia, Curcuma amada, phytochemical profiles, extractive efficiency, Quantitative study.

1. INTRODUCTION

Curcuma species, commonly known for their medicinal and nutritional properties, are members of the Zingiberaceae family. These species have been utilized in traditional medicine across various cultures, primarily for their anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Among the various species of *Curcuma*, *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada* stand out due to their distinct phytochemical compositions and biological activities (Gurung et al., 2018; Singletary, 2020). The pharmacological efficacy of these species is attributed to the presence of diverse bioactive compounds such as phenolics, flavonoids, alkaloids, terpenoids, and essential oils (Patil et al., 2014).

Phytochemicals, especially phenolic compounds, flavonoids, and alkaloids, have been extensively studied for their role in mitigating oxidative stress and inflammation. Total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) are widely used to assess the potential antioxidant and therapeutic activities of plant extracts (Reddy et al., 2019). These compounds exhibit broad-spectrum biological activity, including anti-inflammatory, antioxidant, and anticancer properties, making them of particular interest in drug development (Mohan et al., 2020).

The extraction efficiency of bioactive compounds is influenced by the choice of solvent, which can vary in polarity and, therefore, its capacity to dissolve different phytochemicals. Ethanol, ethyl acetate, and water are common solvents used in the extraction process, each extracting different ranges of compounds based on their solubility (Khan et al., 2021). The solvent polarity can dictate the quantity and diversity of phytochemicals extracted, and thus the medicinal potential of the extract. Studies have shown that ethanol tends to be more effective for extracting phenolic compounds, while water may be more efficient for extracting polysaccharides and other hydrophilic compounds (Ali et al., 2017; Singh et al., 2019).

The goal of this study is to compare the extractive efficiency and phytochemical profile of *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada* using three different solvents, ethyl acetate, ethanol, and water and to evaluate their respective

total phenolic, flavonoid, and alkaloid contents. By doing so, we aim to identify which species and solvent combinations yield the highest concentrations of these bioactive compounds, providing insights into the most effective extraction methods for future pharmacological applications.

2. MATERIAL AND METHODS

Material

The materials used in this study included a variety of chemical reagents and solvents sourced from reputable companies. Ethyl acetate, ethanol, and distilled water were the primary solvents, with ethyl acetate and ethanol being sourced from Sigma-Aldrich (USA) and Merck (Germany), respectively, while distilled water was obtained from a local supplier. Phytochemical screening tests were conducted using specific reagents, such as Wagner's and Hager's Reagents (Sigma-Aldrich), Lead acetate and Copper acetate (Sigma-Aldrich), and Ferric chloride and Folin Ciocalteu reagent (Sigma-Aldrich). Additionally, Benedict's and Fehling's solutions were sourced from Merck (Germany), while Gelatin and Salkowski reagents were also obtained from Sigma-Aldrich. These chemicals were essential for performing the extraction and screening processes that assessed the phytochemical content of the Curcuma species under study.

Procurement of plant materials

Rhizomes of *Curcuma zedoaria*, *Curcuma caesia* and *Curcuma amada* were collected from ruler area of Bhopal, month of February, 2023. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant. Rhizomes of *Curcuma zedoaria*, *Curcuma caesia* and *Curcuma amada* were shade dried at room temperature. The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

Extraction by maceration process

Maceration is a traditional and widely used extraction technique for isolating bioactive compounds from plant materials. This method is particularly suited for extracting phytochemicals from plants with delicate compounds that may be degraded by more aggressive extraction methods involving heat or solvents. Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

Defatting with petroleum ether

40 gram of *Curcuma zedoaria*, 50 gram *Curcuma caesia* and *Curcuma amada* shade dried plant material was coarsely powdered and subjected to extraction with hexane by maceration. The extraction was continued till the defatting of the material had taken place.

Successive extraction with different solvent

Defatted dried powdered has been extracted with different solvent like ethyl acetate, ethanol and aqueous using maceration process for 48 hrs. The mixture occasionally stirred to enhance the extraction efficiency by increasing the contact between the plant material and the solvent. After the soaking period, the mixture is filtered to separate the liquid extract from the solid plant residues. The filtrate contains the dissolved phytochemicals. The solvent is then removed, usually by evaporation under reduced pressure or using a rotary evaporator, to concentrate the extract. The concentrated extract can be further processed or analyzed to isolate specific bioactive compounds.

Determination of extractive value (% yield)

The % yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Qualitative phytochemical analysis is a important process in the field of natural products Chemistry and Pharmacognosy. It involves the identification of the various bioactive compounds present in plant materials. These compounds, known as phytochemicals, include alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds.

3. QUANTITATIVE STUDIES OF PHYTOCONSTITUENTS

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25 µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25 µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of flavonoids. Two ml of 2% $AlCl_3$ solution was added to 2 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

10 mg extract was dissolved in 10 ml of 2N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform. Accurately measured aliquots (40, 60, 80, 100 and 120 µg/ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform.

The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer against the blank prepared as above but without Atropine (Ajanal et al., 2012).

4. RESULTS AND DISCUSSION

The present investigation compared the phytochemical profiles and extractive efficiencies of *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada* using three solvents of varying polarities, ethyl acetate, ethanol, and water. As shown in Table 1, the aqueous extract yielded the highest percentage in *C. caesia* (1.94%) and *C. amada* (2.28%), suggesting a predominance of water-soluble compounds in these species. In contrast, *C. zedoaria* yielded the highest extract with ethanol (1.36%), indicating that it possesses more ethanol-soluble secondary metabolites.

Phytochemical screening revealed significant variation in the type and distribution of bioactive compounds across species and solvents. In *C. zedoaria*, the ethanolic extract tested positive for alkaloids, flavonoids, phenols, and proteins, while the aqueous extract was rich in carbohydrates, saponins, tannins, and sterols **(Table 2).** The ethyl acetate extract was the least diverse, containing only flavonoids. This highlights ethanol as the most efficient solvent for extracting a broad range of compounds from *C. zedoaria*.

Curcuma caesia demonstrated superior phytochemical diversity in its ethanolic extract, which was positive for alkaloids, flavonoids, diterpenes, phenols, and carbohydrates (Table 3). Its aqueous extract was also chemically rich, containing saponins, tannins, proteins, and carbohydrates, while the ethyl acetate extract had a limited but notable presence of flavonoids and sterols. These findings position *C. caesia* as the most chemically versatile species among the three studied.

In the case of *C. amada*, the ethanolic extract showed the presence of alkaloids, flavonoids, diterpenes, and phenols, indicating considerable phytopharmacological potential (Table 4). The aqueous extract, although limited in diversity, still contained flavonoids, phenols, saponins, and proteins, aligning with its high extractive yield in water. Ethyl acetate extracted mainly flavonoids and diterpenes but was otherwise weak in chemical diversity.

Quantitative estimation further confirmed these findings. As presented in Table 5, the ethanolic extract of *C. caesia* exhibited the highest total phenolic (2.56 mg/100 mg GAE) and flavonoid (2.97 mg/100 mg QE) contents, followed closely by *C. amada* (1.58 mg GAE and 3.33 mg QE). Interestingly, *C. amada* surpassed other species in alkaloid content (1.43 mg/100 mg AE), while *C. zedoaria* showed moderate levels of all three constituents, with phenolic and alkaloid contents of 1.34 mg GAE and 0.78 mg AE, respectively.

These results suggest that *Curcuma caesia* is the most potent antioxidant-rich species due to its high phenolic and flavonoid levels, while *Curcuma amada* may be more promising for alkaloid-associated pharmacological effects, including anti-inflammatory and antimicrobial activity. *Curcuma zedoaria*, despite showing lower phytochemical diversity, exhibited decent extractive efficiency with ethanol and may be selectively potent against microbial strains, as discussed in other parts of the study.

Table 1: Extractive values of *Curcuma zedoaria, Curcuma caesia* and *Curcuma amada*

S. No.	Extracts	% Yield* (w/w)		
		Curcuma zedoaria	***Curcuma caesia***	***Curcuma amada***
1.	Ethyl acetate	0.32	1.82	1.56
2.	Ethanolic	1.36	0.77	1.02
3.	Aqueous	1.13	1.94	2.28

Table 2: Result of phytochemical screening of extract of *Curcuma zedoaria*

S. No.	Constituents	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**			
	Wagner's Test:	-ve	+ve	+ve
	Hager's Test:	-ve	+ve	+ve
2.	**Glycosides**			
	Conc. H_2SO_4 Test:	-ve	-ve	-ve
3.	**Flavonoids**			
	Lead acetate Test:	+ve	+ve	+ve
	Alkaline test:	+ve	+ve	-ve
4.	**Diterpenes**			
	Copper acetate Test:	-ve	-ve	+ve
5.	**Phenol**			
	Ferric Chloride Test:	-ve	-ve	-ve
	Folin Ciocalteu Test:	+ve	+ve	+ve
6.	**Proteins**			
	Xanthoproteic Test:	-ve	+ve	-ve
7.	**Carbohydrate**			
	Fehling's Test:	-ve	-ve	+ve
	Benedict's Test	-ve	-ve	+ve
8.	**Saponins**			
	Froth Test:	-ve	-ve	+ve
9.	**Tannins**			
	Gelatin test:	-ve	-ve	+ve
10.	**Sterols**			
	Salkowski Test:	-ve	-ve	+ve

+ve= present, -ve=negative

Table 3: Result of phytochemical screening of extract of *Curcuma caesia*

S. No.	Constituents	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**			
	Wagner's Test:	-ve	+ve	-ve
	Hager's Test:	-ve	+ve	-ve
2.	**Glycosides**			
	Conc. H_2SO_4 Test:	-ve	-ve	-ve
3.	**Flavonoids**			
	Lead acetate Test:	+ve	+ve	+ve
	Alkaline test:	+ve	-ve	-ve
4.	**Diterpenes**			
	Copper acetate Test:	-ve	+ve	+ve
5.	**Phenol**			
	Ferric Chloride Test:	-ve	+ve	-ve
	Folin Ciocalteu Test:	+ve	+ve	+ve
6.	**Proteins**			
	Xanthoproteic Test:	-ve	-ve	+ve
7.	**Carbohydrate**			
	Fehling's Test:	-ve	+ve	+ve
	Benedict's Test	-ve	-ve	-ve
8.	**Saponins**			
	Froth Test:	-ve	-ve	+ve
9.	**Tannins**			
	Gelatin test:	-ve	-ve	+ve
10.	**Sterols**			
	Salkowski Test:	+ve	-ve	-ve

+ve= present, -ve=negative

Table 4: Result of phytochemical screening of extract of *Curcuma amada*

S. No.	Constituents	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**			
	Wagner's Test:	-ve	+ve	-ve
	Hager's Test:	-ve	+ve	-ve
2.	**Glycosides**			
	Conc. H_2SO_4 Test:	-ve	-ve	-ve
3.	**Flavonoids**			
	Lead acetate Test:	+ve	+ve	+ve
	Alkaline test:	+ve	-ve	-ve
4.	**Diterpenes**			
	Copper acetate Test:	+ve	+ve	-ve
5.	**Phenol**			
	Ferric Chloride Test:	-ve	+ve	-ve
	Folin Ciocalteu Test:	+ve	+ve	+ve
6.	**Proteins**			
	Xanthoproteic Test:	-ve	-ve	+ve
7.	**Carbohydrate**			
	Fehling's Test:	-ve	-ve	-ve
	Benedict's Test	-ve	-ve	-ve
8.	**Saponins**			
	Froth Test:	-ve	-ve	+ve
9.	**Tannins**			
	Gelatin test:	-ve	-ve	-ve
10.	**Sterols**			
	Salkowski Test:	-ve	-ve	-ve

+ve= present, -ve=negative

Table 5: Estimation of total phenol and flavonoids content

Curcuma zedoaria				
S. No.	**Extracts**	**Total phenol content (GAE mg/ 100 mg)**	**Total flavonoids Content (QE mg/ 100 mg)**	**Total alkaloids Content (AE, mg/ 100 mg)**
1.	Ethyl acetate	0.40	0.16	0.11
2.	Ethanolic	1.34	0.76	0.78
3.	Aqueous	0.93	0.40	0.52
Curcuma caesia				
1.	Ethyl acetate	0.80	0.15	0.03
2.	Ethanolic	2.56	2.97	1.26
3.	Aqueous	1.17	0.33	0.31
Curcuma amada				
1.	Ethyl acetate	0.52	0.22	0.2
2.	Ethanolic	1.58	3.33	1.43
3.	Aqueous	1.00	0.25	0.56

GAE- Gallic acid equivalent, QE – Quercetin Equivalent, AE – Atropine Equivalent

5. CONCLUSION

This study compared the extractive efficiency and phytochemical content of *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada* using ethyl acetate, ethanol, and water. *Curcuma caesia* exhibited the highest phenolic and flavonoid content, making it the most antioxidant-rich species, while *Curcuma amada* had the highest alkaloid content, indicating strong pharmacological potential. *Curcuma zedoaria* showed moderate levels of all compounds, with ethanol proving the most effective solvent for extracting bioactive substances. These findings highlight the distinct chemical profiles of each species, suggesting their suitability for specific therapeutic applications.

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