Influence of extraction method and solvent on flavonoids and total phenolic content of *Couroupita guianensis* leaves extract

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Abstract

It is well known that the phytochemical composition of a plant extract varies with the extraction conditions. In the present study, the effect of extraction method and solvent on flavonoids, total phenolic content, and phytochemical composition of *Couroupita guianensis* leaves extracts was investigated. Water, 50% water-methanol, and methanol extracts of leaves powder were prepared using maceration and ultrasonic-assisted extraction methods under the same condition of sample to solvent ratio and temperature. The extracts were screened for common phytochemicals like alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and diterpenoids. Flavonoids and total phenolic content in the extracts were determined using the spectrophotometric method. The results reveal that the phytochemical composition of *Couroupita guianensis* leaves extracts was significantly varied with the extraction method and solvent used for extraction. The ultrasonic-assisted extraction method was found to be the best method as compared to maceration for the extraction of phytochemicals from *Couroupita guianensis* leaves.

***Keywords*:** *Couroupita guianensis*, phytochemicals, extraction

1. Introduction

Couroupita guianensis is a medicinal tree belonging to the family Lecythidaceae and it is commonly known cannonball tree. The fruit, bark, leaves, and flowers were used for the treatment of various health disorders. Golatkar et. al. (2001), Elumalai et al., (2012). Janakiraman et.al., (2012) reported that *Couroupita guianensis* leaves were used for the treatment of skin diseases, stomach ache, antithrombotic, antiulcer, antiarthritic activity, and vasodilator activity. The phytochemical constituents of *Couroupita guianensis* extracts are attributed to their medicinal properties. The phytochemicals like alkaloids, phenolic compounds, flavonoids, tannins, terpenoids, saponins, glycosides, phlobatannins, steroids, tannins, and terpenoids in leaves extract of *Couroupita guianensis* were reported by Rreetika et.al., (2015) and Alagesaboopathi (2013). Reethika sing et. al., (2015) prepared the aqueous leaf extract using the soxhlet method and screened for phytochemicals. They reported the presence of alkaloids and the absence of saponins in aqueous leaf extracts of *Couroupita guianensis*. On the other hand, Alagesaboopathi (2013) reported quite an opposite result, the presence of saponins and the absence of alkaloids in aqueous leaf extract of *Couroupita guianensis*. The reports showed that the phytochemical constituents of *Couroupita guianensis* leaf extract vary from plant to plant.

Hasmida et.al., (2014), Ana Bucic et.al., (2011), and Karimi et.al., (2015) reported that the phytochemical composition of any plant extracts is highly dependent on the extraction conditions like a solvent, ratio of solvent to plant material, the particle size of the plant material, extraction temperature, extraction time and extraction methods. El Akrem et.al., (2007), Rebey et. al., (2012), and Cheok et.al., (2012) were observed that solvent and extraction methods are the main factors affecting the phytochemical composition of plant extracts. Studies on phytochemical screening of different plant extracts namely *Cuminum cyminum* L. seed, *Garcinia mangostana* Linn hull, *Quercus coccifera*, and *Juniperus phoenicea* fruits were carried out to select the appropriate solvent and extraction method. However, the effect of solvent and extraction method on the phytochemical composition

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of *Couroupita guianensis* leaf extracts was not reported so far in the literature. Numerous studies showed that the antioxidant capacity and antibacterial activity of plant material typically results from secondary metabolites namely, phenolic compounds, and flavonoids. Therefore, in the present work, the influence of solvent and extraction method on the phytochemical composition of *Couroupita guianensis* leaves extracts was investigated to predict the proper solvent and extraction method for effective extraction of phenolic compounds and flavonoids from *Couroupita guianensis* leaves.

2. Materials and methods

2.1. Collection and preparation of plant material

Fresh and disease-free leaves of *Couropita guianensis* were collected from the botanical garden of Andhra Loyola college campus, Vijayawada, Andhra Pradesh, India. The leaves were dried under shade and powdered with the help of a domestic grinder. The powdered leaves were stored at 4ºC and used for further analysis.

2.2. Preparation of *Couropita guianensis* leaves extracts

The polarities of the phytochemicals range from polar to nonpolar. In the present work, only polar solvents like water, 50% water-methanol, and methanol were selected for extraction. Water and methanol are commonly used for the extraction of phytochemicals from plant materials. Aqueous, 50% water-methanol, and methanol extracts of *Couropita guianensis* leaves were prepared using two different extraction methods namely maceration (MCR) and ultrasound-assisted extraction (UAE). The extraction was conducted at room temperature for both MCR and UAE.

Phytochemicals from *Couropita guianensis* leaves were extracted using Maceration (MCR) as follows, exactly 1.0 gram of leaves powder was extracted with 50 ml of water, 50% water-methanol, and methanol in three different conical flasks of 250 ml capacity. The flasks were sealed with aluminum foil to prevent evaporation of solvent during extraction. The contents were left for 24 hours at room temperature and then centrifuged at 5000 rpm/min for 10 min. The extracts were screened for the phytochemicals.

Ultrasonic Assisted Extraction (UAE) process was carried out using Labman, a Digital ultrasonic cleaner. Exactly 1.0 gram of leaves powder was extracted with 50 ml of solvents namely water, 50% water-methanol, and methanol, separately. A 250 ml round bottom flask containing leaves powder and solvent was kept in an ultrasonic bath for 2 hours at the temperature of 30ºC. After extraction, the contents were centrifuged at 5000 rpm/min for 10 min. The extracts were analyzed for the phytochemicals.

2.3. Preliminary phytochemical screening

Aqueous, 50% water-methanol, and methanol extracts of *Couropita guianensis* leaves were subjected to preliminary phytochemical screening for various phytochemical constituents in the leaves extracts. The phytochemical analysis for alkaloids, phenolic compounds, flavonoids, terpenoids, diterpenoids was carried out by following a standard procedure reported in the literature, Raman (2006) and Harborne (2005).

Test for Alkaloids

Wagner s test: To 1 ml of the extract few drops of Wagner's reagent were added. The yellow formation or brown precipitate confirms the presence of alkaloids.

Mayer s test: Exactly 1 ml of extract was taken in a test tube to which 1 ml of Mayer s reagent was added. The formation of yellowish buff-colored precipitate infers the presence of alkaloids.

Dargen Dorff's Test: To 1ml of the extract few drops of Dargen Dorff's reagent (solution of Potassium Bismuth Iodide) was added. The formation of a red precipitate indicates the presence of alkaloids.

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Test for phenolic compounds

Ferric chloride Reagent Test: To 1.0 ml of the extract, a few drops of 5% aqueous ferric chloride solution was added. The formation of an intense color precipitate showed the presence of phenolic compounds.

Test for Flavonoids

Shinoda's test: In a test tube containing 0.5 ml of extract, 6-10 drops of concentrated HCl and a small piece of magnesium were added and the solution was boiled for a few minutes. The development of reddish-pink color indicates the presence of flavonoids.

Test for terpenoids

Exactly 2 ml of extract was mixed with 2 ml of chloroform and then 3 ml of concentrated sulphuric acid was added carefully from the sides of the test tube. The appearance of a reddish-brown color layer at the interface of two liquid layers indicates a positive result for terpenoids.

Test for diterpenoids

To 1.0 ml of the extract a few drops of 1% aqueous copper acetate solution were added. The formation of emerald green precipitate confirms the presence of diterpenoids.

2.4. Determination of total phenolic compounds and flavonoids content

The total phenolic content of leaves extracts was determined by the Folin-Ciocalteu method reported by Singleton et.al., (1999). In brief, exactly 0.1 ml of extract was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent. After 5 min, 2 ml of 20% sodium carbonate solution was added to the solution mixture and incubated for 30 min at room temperature. The absorbance of the solution mixture was measured at 765 nm against a blank on Varian Cary 50 UV-VIS spectrophotometer. Total phenolic content was calculated from calibration curve ($y = 20.5x + 0.045$, $R^2 = 0.996$) and expressed as mg of gallic acid (GAE) equivalents per gram of leaves powder.

The flavonoid content of leaves extracts was determined using a spectrophotometric method reported by Miliauskas et.al., (2004). Exactly 0.5 ml of extract was mixed with 0.5 mL of 5% ethanolic aluminum trichloride solution and 0.5 ml of sodium nitrate solution. After incubation of 10 min, the absorbance of samples was determined at 425 nm against a blank. Varian Cary 50 UV-VIS spectrometer was used to determine the absorbance of the solutions. Flavonoid content of leaves extracts was calculated from calibration curve ($y = 4.838x + 0.001$, $R^2 = 0.999$). The flavonoids content of the extracts was expressed as mg rutin equivalents per gram of leaves powder.

2.5. Statistical analysis

All experiments were done in triplicate and reported. The analysis of total phenolics and flavonoid contents was repeated thrice in an independent manner. Analyzed data were represented as mean±SE.

3.0. Results and discussion

Water, 50% water-methanol, and methanol extracts of *Couroupita guianensis* leaves were prepared using MCR and UAE methods under similar conditions of sample to solvent ratio. The extracts were screened for phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, and diterpenoids. Phytochemical analysis results were presented in Table 1. The phytochemical screening results indicate the presence of alkaloids, phenolic compounds, flavonoids, tannins, terpenoids, and diterpenoids. Similar to present results, Manimegalai and

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Rakkimuthu (2012) reported the presence of phenolic compounds, flavonoids, tannins, terpenoids, and alkaloids in water and alcohol extracts of *Couroupita guianensis* leaves and stem. Reetika sing et. al., (2015) reported the presence of flavonoids in water extracts and absence in alcohol extracts, whereas Alagesaboopathi (2013) observed the presence of flavonoids in an alcohol extract of *Couroupita guianensis* leaves. Similarly, the current results showed the absence of flavonoids in 50% water-methanol and methanol extracts. However, flavonoids are observed only at a detectable level in water extracts. In addition, flavonoids were not detected in water extract prepared using the maceration method and only present in the extracts prepared using the UAE method.

The present results showed that the phytochemical composition of *Couroupita guianensis* leaves differs from plant to plant, extraction solvent, and the method employed for extraction. The phytochemicals such as phenolic compounds, tannins, terpenoids, and diterpenoids in the extracts were highly dependent on the nature of the solvent used for extraction, as presented in Table 1. A higher level of phenolic compounds was observed in 50% water-methanol extract while tannins, terpenoids, and diterpenoids were observed in the water extract. In addition, all the phytochemicals were found to be higher in UAE extracts than MCR. The high efficiency of the UAE method is due to the disruption of the plant cell wall by the ultrasonic waves, which facilitates the release of phytochemicals in the solvent during extraction. In the case of maceration, the phytochemicals are released into the solvent by diffusion which is a slow process.

Total phenolic compounds and flavonoids content in *Couroupita guianensis* leaves extracts were determined under similar conditions of solvent to solid ratio. The variation of total phenolic compounds and flavonoids content with extraction method and the solvent was presented in Figures 2 and 3, respectively. The total phenolic content of *Couroupita guianensis* leaves was higher in UAE extracts of 50% water-methanol (4.516 mg GAE/g) followed by methanol (4.10 mg GAE/g) and water (3.64 mg GAE/g). From figure 3 it was clear that a slightly higher concentration of flavonoids was observed in UAE extracts of water (2.07 mg RE/g) followed by 50% water-methanol (1.98 mg RE/g) and methanol (1.89 mg RE/g). UAE method was found to be the best method as compared to maceration for extraction of both phenolic compounds and flavonoids from *Couroupita guianensis* leaves. This difference is due to differences in extraction mechanisms.

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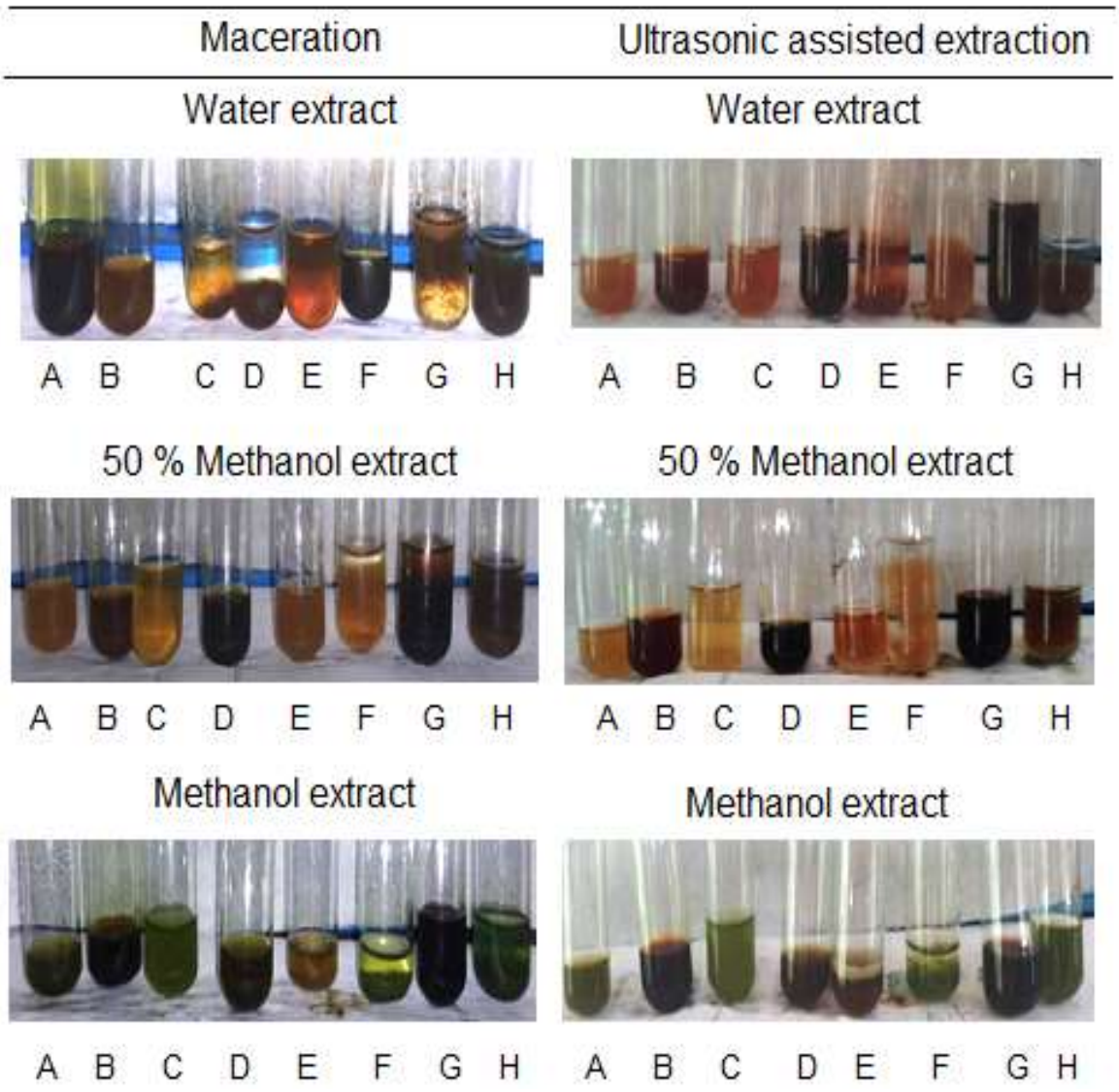

Note: Alkaloids (A, B, C), Phenolics (D), Flavonoids(E), Tannins(F), Terpenoids (G), Diterpenoids (H)

Figure 1. Phytochemical screening of *Courpoita guianensis* leaves extracts

Table 1. Phytochemical analysis of *Courpoita guianensis* leaves extract

Phytochemical	**Solvent**	**Extraction method**	
Alkaloids		**MCR**	**UAE**

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Dargen Dorff's test	Water	+	+
	50% Methanol	-	-
	Methanol	-	-
Mayer s test	Water	+	+
	50% Methanol	-	-
	Methanol	-	-
Wagner s test	Water	+	+
	50% Methanol	-	-
	Methanol	-	-
Phenolics	Water	+	+++
	50% Methanol	++	+++
	Methanol	++	++
Flavonoids	Water	+	++
	50% Methanol	-	-
	Methanol	-	-
Tannins	Water	+++	+++
	50% Methanol	+++	++
	Methanol	+	+
Terpenoids	Water	++	+++
	50% Methanol	++	++
	Methanol	-	+
Diterpenoids	Water	++	+++
	50% Methanol	++	++
	Methanol	+	+

Note: Strongly present (+++), Moderately present (++), Present (+), Absent (-).

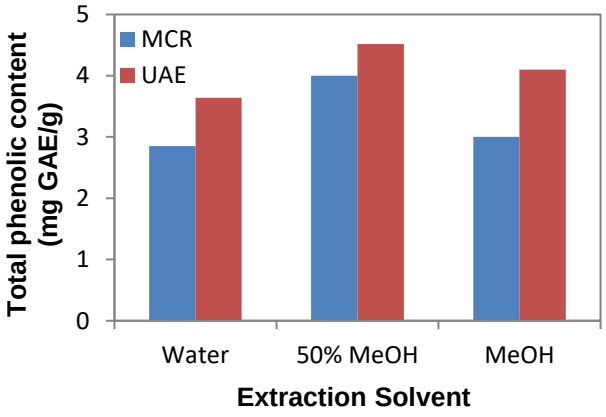

Figure 2. Variation of total phenolic content with extraction method and solvent.

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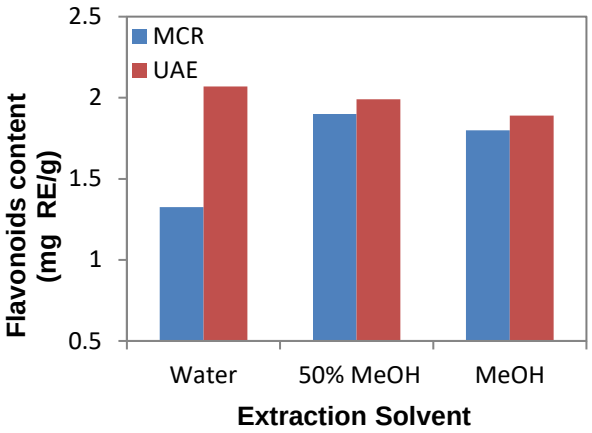

Figure 3. Variation of flavonoids content with extraction method and solvent.

4.0. Conclusions

Effect of solvent and extraction method on the phytochemical composition of *Couroupita guianensis* leaves extracts was investigated. Results of the present study infer that the nature of the solvent (polarity of solvent) and extraction method (extraction mechanism) significantly influences the phytochemical content in the *Couroupita guianensis* leaves extracts. The results showed that 50% aqueous-methanol was proved to be the best solvent and the UAE method was the best method for extraction of phenolic compounds and flavonoids from *Couroupita guianensis* leaves. The present results suggest the optimum extraction conditions for efficient extraction of phytochemicals from *Couroupita guianensis* leaves.

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