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Comparing Three Extractive Solvents for Phytochemicals in *Citrullus colocynth* Relevant for Polycystic Ovarian

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ABSTRACT

Background and Purpose: The choice of extraction solvents often influences the efficacy and potency of herbal medicinal extracts. *Citrullus colocynthis* (CCT) fruit extract has been used in the management of various pathological conditions, particularly polycystic ovarian syndrome (PCOS) due to numerous phytochemical constituents such as terpenoids and flavonoids. This study investigated the qualitative and quantitative profiles of phytochemicals in CCT using three different extraction solvents: aqueous, *omidun* (a local extraction medium), and ethanol.

Methods: CCT fruits were procured, washed and cut into pieces. Equal amounts were extracted in equal volumes of water, *omidun*, and ethanol. The bulk yield, class of phytochemicals, and the quantities of each class were determined using standard methods.

Results: The extractive yield for each solvent was 50.4, 39.3, and 10.3%w/w for aqueous, *omidun*, and ethanol respectively. Preliminary screening to determine the phytochemicals that should be quantified revealed that tannins and flavonoids were present in all three solvent extracts, but alkaloids were present only in ethanol extract, triterpenoids only in *omidun* and saponins were present in aqueous and ethanol extracts. *Omidun* was the most efficient in extracting flavonoids (73.40 mg/g), steroids (52.53 mg/g), and triterpenoids (76.45 mg/g).

Conclusion: Of the three solvents, o*midun* was the most efficient in extracting the phytochemicals in CCT fruit that have been reported to alleviate infertility indices of PCOS, hence should be considered as the extraction medium for this purpose.

INTRODUCTION

Medicinal plants play essential roles in pharmacological studies due to their active phytochemical contents and serve as raw materials in drug development (Al-Snafi, 2015). The promising therapeutic effects of medicinal plants have led to calls for their application in the treatment of various diseases. According to the World Health Organisation, 70% of the world population, particularly in developing countries, depends on medicinal plants (WHO, 2018).

Phytochemicals like terpenoids, polyphenols, isoprenoids, flavonoids, saponins, dietary fibers, and specific polysaccharides have been documented to exhibit strong antioxidant activity and demonstrate antimicrobial, anthelmintic, antiallergic, antispasmodic, and antiviral properties (Rodríguez-Negrete *et al*., 2024). Additionally, they contribute to the regulation of body hormones, enhance systematic body functions and provide protective effects in many pathological conditions (Rowles *et al*., 2020; Jiang *et al*., 2018). Phytochemicals are now extensively employed in the formulation of functional foods, drugs and nutraceuticals due to increased emphasis on translational research.

Citrullus colocynthis (CCT) (Figure 1) is commonly referred to as bitter apple, bitter cucumber and "*egusi bara*" by the Yoruba tribe of Nigeria. It is a desert viny plant, native to the Mediterranean Basin and West Asia, and the Levant (Ali-Shlayeh *et al*., 2016). It resembles a common watermelon vine but bears small hard fruits with a bitter pulp. It originally bore the scientific name *Colocynthis citrullus* of Cucurbitaceae family (Bhasin *et al.,* 2020). CCT fruit and seeds have been proven to possess some pharmacological qualities such as laxative and purgative, antidiabetic, anti-inflammatory, and antioxidant effects (Errairaji *et al*., 2010; Bhasin *et al*., 2020; Shreyasi *et al*., 2023). Additionally, it has fertility enhancing effects particularly in polycystic ovarian syndrome (PCOS) and ovarian hyperandrogenism (Diamant- Kandaki, 2008).

Figure 1: *Citrullus colocynthis* fruits.

The possible effects of CCT on hormonal levels and folliculogenesis in PCOS-induced experimental models have been reported. Mohammad *et al.* (2017) highlighted the efficacy of CCT in regulating hormones and folliculogenesis which are the fundamental processes for female reproduction. These effects are associated with its reducing sugars and steroid contents amongst others (Barzegar *et al.,* 2022; Shreyasi *et al*., 2023). Flavonoids have been reported to improve insulin sensitivity, modulate steroidogenic enzymes, and restore ovarian function which are well implicated in PCOS (Mohammad *et al*., 2017). Terpenes have been directly linked to PCOS by modulating insulin signalling and improving glucose metabolism (Khezerlou and Jafari, 2020). In an animal study, terpenes such as lupeol restored endocrine dysregulations leading to normal ovarian histomorphology, improved folliculogenesis and reduction in androgen level (Rezaei-Golmisheh *et al.,* 2024). Some terpene-rich compounds have been shown to influence steroidogenesis, thereby helping to balance estrogen and androgen secretions and mitigate metabolic, hormonal, and reproductive dysfunctions in PCOS (Esmaeilzadeh *et al.,* 2022).

Solvent extraction is the most widely used technique for extracting terpenes and other phytoconstituents from plants like CCT fruits (Stephane *et al.,* 2021). The choice of solvent is crucial in determining the quantity of phytochemicals in extracts. Extraction solvents should have low toxicity and strong preservation capabilities, promote extract absorption, should be evaporated at low temperatures, and be devoid of dissociation and complexation with extracts. Also, the extraction speed, and potential health concerns of extracts should all be considered when choosing a solvent (Das *et al.*, 2010; Yolci *et al*., 2019). Conventional techniques include microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed electric field extraction (PEF), vibro-cavitation extraction, vacuum-oscillating boiling extraction, mill-based extraction, rotation-planar extraction (RPE), liquid-gas extraction (LGS), enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), and natural deep eutectic solvent extraction (NADES) (Shikov *et al*., 2022; Quiterio *et al*., 2022).

Omidun, the supernatant of fermenting maize slurry (*ogi* in Yourba), is the preferred solvent for extraction in the southwestern region of Nigeria. Pap water (*omi ogi* or *omidun* in Yoruba language, and *nmiri akamu* in Igbo language of Nigeria), is gotten by filtering out water from ground maize that has been left to ferment. It contains the gut-friendly bacterium *Lactobacillus acidophilus* (Idebi *et al.,* 2024). It also contains phenolic acids, flavonoids, carotenes and dietary fibers which reinforce its medicinal usefulness (Falana *et al.,* 2016; Afolayan *et al*., 2017). Different tribes in Nigeria make use of *omidun* as the

preferred solvent for extraction of herbs for treating conditions like female-related infertility. This study compared the efficiencies of three extraction solvents (aqueous, *omidun* and ethanol), on the phytochemical yields from CCT fruit with a view to recommending the best for the treatment of PCOS.

MATERIALS AND METHODS

Plant Material

Citrullus colocynthis (CCT) fruits were procured from Mushin market, Lagos, Nigeria. The fruits were identified at the Department of Taxonomy, University of Lagos, Nigeria, by Mr. Nodza George who a taxonomist, and the herbarium number 100404 was assigned to it. Ethical approval referenced CMUL/ACUREC/04/25/1870 was obtained from the research and ethics committee of the College of Medicine, University of Lagos.

Preparation of Aqueous, Omidun and Ethanol extracts

The fruits were washed, weighed, chopped into pieces and three equal portions, each of 1.25 kg was soaked in 2.5 L each of distilled water (aqueous), *omidun*, and ethanol (80%v/v). The mixtures were left for 48 h and then filtered using a sieve. The resulting filtrates were placed in a Gallenkamp oven to dry (Abubakar and Haque, 2020). The yields were 50.4%w/w, 39.3%w/w, 10.3%w/w, and for aqueous, *omidun,* and ethanol and medium respectively.

Qualitative Analysis

Detection of phenols, tannins and flavonoids

Phenol and tannins were detected using ferric chloride test (Sofowora, 2008; Sati *et al*, 2015). The lead acetate test (Sofowora, 2008) was used for detecting the presence of flavonoids.

Detection of alkaloids

The presence of alkaloids was detected using Wagner's test (Archana *et al*, 2012).

Detection of steroids

Libermann Burchard's test and Hesse's response were used to confirm the presence of steroids. (Harborne, 1998).

Detection of saponins

Froth test was used to detect the presence of saponins in the extracts (Sofowora, 2008).

Detection for terpenoids

The "Salkowski test for detection of terpenoids" was used (Indumathi *et al.,* 2014).

Quantitative Analysis

Phenols

The Folin-Ciocalteu method as originally described by Pearson (1979) and described by Ainsworth and Gillespie (2007) and absorbance at 765 nm was measured in triplicates using spectrophotometer at a wavelength of 765 nm.

Tannins

The method described by Makkar *et al.* (1993) was used and absorbance of each sample (extracts and standard tannin solutions) was measured at 765 nm using a spectrophotometer.

Flavonoids

The aluminum chloride colorimetric method was used to estimate flavonoid content, using quercetin as the standard, and at the wavelength of 415 nm (Chang *et al*., 2002).

Alkaloids

Total alkaloid content of each extract was measured using method as described by Shamsa *et al.* (2007). Atropine was used as the standard and wavelength of measurement was 470 nm.

Alkaloids

Total alkaloid content of each extract was measured using method as described by Shamsa *et al.* (2007). Atropine was used as the standard and wavelength of measurement was 470 nm.

Steroids

The total steroids in each of the extracts was measured using methods as described by Salomi *et al.* (2019) at the wavelength of 780 nm with cholesterol as the standard.

Saponins

The determination of the total saponin content in the extracts was made according to the vanillin-sulfuric acid colorimetric method (Akinseye *et al*., 2017) using diosgenin as the standard. The absorbance of the solution was read at 544 nm.

Terpenoids

The Malik *et al.* (2017) method was used to quantify the terpenoids in each of the extracts, by evaporating to dryness under reduced pressure.

RESULTS

Presence of Classes of Phytochemicals

None of the solvents was efficient in extracting all the phytochemicals in CCT (Table 1). While alkaloids, steroids and triterpenoids were not detected in the aqueous extract; alkaloids and saponins were not detected in the *omidun*; and steroids and triterpenoids were not detected in the ethanol extract. The results were the basis for the quantitative experiments.

Quantities of Phytochemicals

Table 2 shows that ethanol was most efficient in extracting the tannins (97.00 mg/g CCT), followed by *omidun* (63.83

mg/g CCT) and water (38.65 mg/g CCT). *Omidun* extracted flavonoids the most (73.40 mg/g CCT), followed by ethanol (60.00 mg/g CCT) and aqueous was the least (42.20 mg/g CCT). Alkaloids were quantified only in ethanol (38.00 mg/g CCT) while steroids and triterpenoids were quantified only in *omidun* (76.45 mg/g). Saponins were quantified in aqueous (1.00 mg/g CCT) and ethanol (18.00 mg/g CCT) solvents.

DISCUSSION

This present study provides valuable insights into the suitable extraction solvent for CCT particularly targeting phytochemicals reported to possess anti-PCOS potential. *Omidun* has since been used in traditional medicine as a solvent for extraction of medicinal plants. This study showed that *omidun* extract had the highest quantity of flavonoids and steroids, and only the solvent that had terpenoids and flavonoids which are phytochemicals that have been reported to alleviate PCOS (Mohammad *et al.*, 2017) when compared with the aqueous and ethanol extracts. Scientific evidence has proven *omidun* to be suitable for herbal extraction because of its lactic acid content that effectively allows it to extract bioactive compounds from plant materials (Afolayan *et al.,* 2017). The high yields of phytochemicals in *omidun* when compared with the aqueous and ethanol solvents indicate that it has a superior extractive capacity which may be associated with high affinity and ability to dissolve flavonoids and triterpenoids (Falana *et al.,* 2016).

Table 1: Phytochemicals present in the three solvents used for the extraction of CCT fruit

Phytochemicals	Aqueous	*Omidun*	Ethanol
Tannins	+	+	+
Flavonoids	+	+	+
Alkaloids	-	-	+
Steroids	-	+	-
Saponins	+	-	+
Triterpenoids	-	+	-

Table 2: Mean quantities of the various phytochemicals in the CCT extracts by each of the three solvents

	Aqueous (mg/g)	*Omidun* (mg/g)	Ethanol (mg/g)
Tannins	38.65	63.83	97.00
Flavonoids	42.20	73.40	60.00
Alkaloids	-	-	38.00
Steroids	-	52.53	-
Saponins	1.00	-	18.00
Triterpenoids	-	76.45	-

Values are the means of two measures for each phytochemical.

Distilled water is widely used as solvent to extract highly polar molecules such as organic acids and alkaloids because it is cheap, non-toxic, non-flammable and has the capacity to dissolve phytochemicals. Its drawbacks include increasing bacterial or mold growth, difficult preservation of extract, near zero interaction with non-polar or lipophilic compounds due to high polarity and high boiling point which causes hydrolysis and high concentration time and heat, unlike polar hydrophilic organic solvents like ethanol that is miscible with water, aids the extraction of polar secondary compounds but requires small amount of heat to concentrate (Abubakar and Haque, 2020).

In a study by Munira *et al*. (2020) on different extraction solvents on *Picramnia bullata*, a woody medicinal plant is in conformity with the findings from this present study. In that study, there were fewer non-polar compounds extracted with water when compared with organic solvents. The aqueous extract did not contain non-polar compounds such as alkaloids or lipophilic terpenes which were present when other organic solvents such as ethanol were used (Lim *et al.,* 2007). Many bioactive compounds are not very soluble in water and may not be substantially extracted in an aqueous medium. Additionally, diffusivity and tissue penetration are compounding factors. Higher surface tension, viscosity, and poor penetration into hydrophobic regions of membranes, may be responsible for the absence of terpenoids in the aqueous extract of CCT (El-Gamou *et al.,* 2022).

Ethanol has been reported to selectively solubilize polar compounds such as flavonoids, phenolics, and glycosides while underrepresenting non-polar constituents like terpenes or sterols (El-Gamou *et al.,* 2022*)*. This affects the overall phytochemical profile and yield as seen in the present study in which terpenes were absent unlike in the *omidun* medium (El-Gamou *et al.,* 2022). Although ethanol is a semi-polar solvent that can dissolve both polar and non-polar components, exposure to heat during preparation may lead to loss through volatility and heat degradation of compounds such as phenols, flavonoids and vitamins (Zhang *et al.,* 2018).

Omidun has become an important indigenous extraction medium in African traditional medicine, for bioactive constituents that are less extractable by water and ethanol. Several ethnobotanical reports have confirmed the use of *omidun* in preparing herbal remedies, where it serves not only as an extraction medium but also as a fermentation enhancer that improves the efficacy of herbal mixtures (Falana *et al*., 2011; Afolayan *et al*., 2017; Omeiza *et al*., 2020). The efficacy of some plant extracts appears to depend on the use of *omidun* as the extracting solvent since *omidun* exhibits antioxidant and anti-inflammatory activities, suggesting synergistic effects between the plant's

organic acids and lactic acid bacteria (LAB) metabolites present in it (Omeiza *et al*., 2020). This present study further supports the findings of Omeiza *et al*. (2020) that *omidun* served as a vehicle that facilitated the extraction of key phytoconstituents possibly due to its lactic acid content".

CONCLUSION

CCT has been documented to possess medicinal properties including alleviation of PCOS. However, the use of a suitable solvent is key in extracting the appropriate bioactive constituents. This present report positions the *omidun* as a more efficient solvent for the extraction of CCT phytochemicals such as terpenoids and flavonoids that are relevant to PCOS treatment.

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AUTHORS' CONTRIBUTION

The conceptualization, analysis, writing of original draft and execution of study was done by FOA and AAB, CB contributed to CCT extraction, data collection and analysis. AAB and AAAO supervised the study. All authors read the manuscript and approved its submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be accepted by them.

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