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GC-FID ANALYSES OF THE PHYTOCHEMICAL CONSTITUENTS OF BOILED WATER EXTRACTS OF THE LEAVES OF *JUSTICIA CARNEA, FICUS CAPENSIS, MUCUNA PRURIENS* AND THEIR COMBINATION.

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Abstract

The decoction of the leaves of *Justicia carnea*, *Ficus capensis* and M*ucuna pruriens* are widely used to treat various diseases including anemia in many communities in Nigeria. In this present study, the phytochemical constituents of the boiled extracts of the leaves of *Justicia carnea*, *Ficus capensis*, M*ucuna pruriens* and their combination was analyzed using gas chromatography linked to flame ionization detector (GC-FID). The results obtained show that the boiled water extract of the leaf of *Justicia carnea*, *Ficus capensis* and *Mucuna pruriens* are rich in bioactive compounds including catechin, kaempferol, tangeretin, silymarin, Quercetin, Luteolin, Retusin, Ellagic acid, Naringenin, Isorhamnetin, Hesperidin and Epicatechin. *F. capensis* has the highest amount of catechin, which was the most abundance among all the phytochemicals present in the extracts. It is present at a concentration of 190.06ppm in the *F. capensis* extract while *J. carnea*, *M. pruriens* and their combination showed 0.57, 1.84 and 0.51ppm respectively. Sylimarin was only found in *J. carnea* extract while Revertrol was absence in *M. pruriens* extract but Present in others. These phytochemicals may be responsible for the various biological activities that has been reported on this plant leaves and their combination had a balance concentration of these phytochemicals and, thus could lead to reduce toxicity.

Keywords: GC-FID, Phytochemical constituents, Boiled water extracts

Introduction

The integration of traditional medicine into the conventional healthcare framework, especially in developing nations like Nigeria, has facilitated dependable access to herbal remedies. This shift has prompted an increased interest in herbal alternatives, as well as the reformulation and innovation of herbal treatments for the management of various diseases, including anemia, diabetes, and microbial infections (Kumadoh *et al*., 2024). The biological activities of these herbs is largely dependent on the type of phytochemicals present in them and the quantity of such phytochemicals in the herbs.

Phytochemicals refer to a diverse array of chemical compounds found in plants, each exhibiting distinct structures and functions. Within the plant kingdom, these compounds fulfill various roles related to protection and reproduction. For instance, they can contribute to coloration and scent, which serve to deter herbivores and attract pollinators. . Furthermore, plants produce antifeedants and toxins to safeguard themselves from insect predation, as well as allelochemicals that provide a defense mechanism against herbivory (Egbuna *et al*., 2018). Additionally, some play a crucial role in defending against pathogens, while some which are hormones function in growth regulation and signaling processes. The disciplines of Pharmacy and Pharmacognosy, along with Complementary and Alternative Medicine, Ethnomedicine, Biochemistry, Microbiology, Bioinformatics, and Computational Chemistry, utilize phytochemical knowledge to facilitate the identification of bioactive compounds.

Phytopharmaceuticals are compounds derived from plants that possess pharmacological properties (Mtewa *et al*., 2021). They constitute a significant portion of medications derived from plant-based molecules rather than synthetic ones. Phytopharmaceuticals are available in various forms such as whole fruits, purees, vegetables, prepacked fruits, vegetable products, and supplements. They play a crucial role in maintaining optimal body functions. The primary mode of action for phytopharmaceuticals involves targeting specific receptors, disrupting disease pathways, and interfering with the life cycles of pathogens (Mtewa *et al*., 2021).

The decoction of the leaves of *Justicia carnea, Ficus capensis* and M*ucuna pruriens* are widely used to treat anemia in many communities in Nigeria. *Justicia carnea* hooker (Figure 1) is called *"Ulogwu di anya*" in some parts of Abia state, Nigeria where the boiled extract of the leaf is used for the treatment and prevention of anemia. Pregnant women, nursing mothers, people with sickle cell anemia and patients suffering from malaria, typhoid and hepatitis also take the boiled extracts of the leaves. The boiled leaves extracts are obtained by boiling a mixture of the leaves in water for a period of 10 to 15 minutes with the resultant appearance of a blood-like colour solution (Moswa *et al.,* 2015). This extract can be taken when hot or it can be allowed to cool before taking. It is used to treat anemia in many parts of Nigeria most especially in Abia and Cross River States of Nigeria. The plant, according to Pius *et al.* (2010), is used as a treatment of anemia in Congo by Jehovah's Witnesses, well known for their refusal of blood transfusion. Phytochemical screening of the plant revealed the presence

of alkaloids and polyphenols such as flavonoids, tannins, leucoanthocyanins, quinones and anthocyanins (Pius *et al.*, 2010).

Ficus capensis (Figure 1), locally called *"akokoro"* or '*akpulu*' in igbo, "*uwaryara"* in hausa, "*opoto*" in Yoruba, "*rimabichehi*" in Fulani and "*obada*" in Edo, belongs to the family Moraceae and has been considered an underutilized plant (Ezeigwe *et al.,* 2020). The leaves of this plant have been found to be abundant in dry season as a result of the plant's resilience, adaptation and tolerance to adverse climatic conditions, making it a good substitute to help with the cases of reduced consumption of green leafy vegetables experienced in the dry seasons. It is one of the plants used in traditional medicine in Nigeria, for treating various diseases and promotes vascular health (Ezeigwe *et al.*, 2020). The leaves of *F. capensis* are commonly used as a vegetable in foods with a substantial blood boosting effect, and possess the ability to prevent the sickling of red blood cells (Ezeigwe *et al.*, 2020). In Nigeria, decoctions and aqueous extract of *F. capensis* are said to be used traditionally in the treatment of anemia, tuberculosis, pains, convulsions and wounds. Oral administration of aqueous extract of *F. capensis* has be reported to increase haemoglobin concentration, packed cell volume and red blood cells of albino rats (Ezeigwe *et al.*, 2020).

Mucuna pruriens (Figure 1) is a vegetable plant native to tropical and subtropical regions in Africa, South America, and Asia. It is part of the Fabaceae family and is one of many species of *Mucuna.* It is commonly referred to as 'velvet bean' or 'cowhage' (Pathania *et al.,* 2020). The plant is notorious for its extreme itches and skin irritation, particularly the young foliage and seed pods. When in contact with the skin, it produces severe irritations and many medium-sized red swollen areas on the skin that are actively itchy (Barde *et al.*, 2023). It has been traditionally used in Ayurvedic medicine for the treatment of a wide range of ailments including anemia. In the south-eastern geopolitical zone of Nigeria *M. pruriens* is popularly known as '*agbala*', and '*karara*' in the Hausa language speaking region (Barde *et al.*, 2023). The leaf is squeezed with water and drunk to treat anemia. Others boil the leaf in water and drink it for the treatment of anemia.

Numerous scientific investigations have been conducted regarding herb-drug interactions; however, there is a notable scarcity of research focused on herb-herb interactions, despite their prevalence in traditional medicinal practices (Deciga-campos *et al*., 2021). Similar to studies on drug-drug and herb-drug interactions, the primary objective of combining medicinal plants is to enhance their therapeutic efficacy while minimizing the required dosages. Additionally, it is crucial to ascertain whether the interactions among natural products can lead to synergistic, additive, or antagonistic therapeutic or toxic effects. Consequently, identifying the optimal proportions that yield the most favorable therapeutic outcomes will facilitate the development of effective phytoformulations. Therefore the aim of this research is to investigate the phytochemical constituents of the boiled extracts of the leaves of *Justicia carnea, Ficus capensis,* M*ucuna pruriens* and their combination.

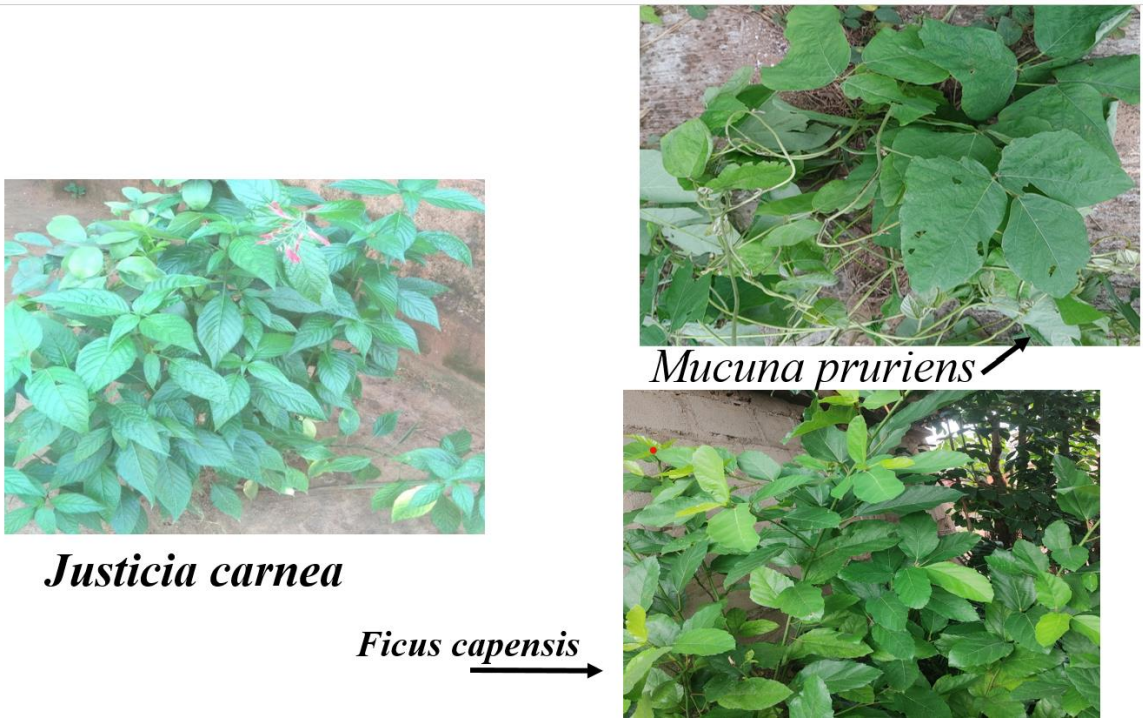

Figure 1. The leaves of *Justicia carnea*, *Ficus capensis* and *Mucuna pruriens*

Materials and Methods

Sample collection

The leaves of *Justicia carnea* and *Ficus capensis* were collected from Prof. S.C. Udedi's Botannical garden, Awka, while the leave of *Mucunna pruriens* was collected from Mr Fidelis Asogwa's farm land at Opi Uno in Nsukka, Enugu State. The leaves were taken to the department of Botany, Nnamdi Azikiwe University for authentication. They were assigned the following herbarium numbers; NAUH32^{B}, NAUH203^{B} and NAUH74^{E} for *Ficus capensis*, *Justicia carnea* and *Mucuna pruriens* respectively.

Sample preparation

The leaves were rinsed with deionized water, drained in a filter basket and then spread to dry in the drying room of Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University Awka. The dried leaves were ground separately using electric blender and the resulting powder were extracted using the method of Barros *et al.* (2007) with slight modification, by boiling 100g of each powder in 1L of deionized water for 10mins. This was filtered using a five layer muslin cloth before filtering with Whatman No 4. Filter papers using a vacuum pump. The resultant filtrate was concentrated using freeze dryer (SP Scientific, USA) and stored at 4^{o}C till further use.

The combined extract was prepared by carefully weighing 0.2g of each of the freeze-dried extract and dissolving them together in 60ml of deionized water to produce a solution of concentration 10mg/ml as described by Barros *et al.* (2007). An aliquot (0.6g) of individual leaf extracts were also separately weighed and dissolved in 60ml of deionized water to give same concentration of 10mg/ml as the

combined extract. These solutions were used for the GC-FID analysis.

Quantification of Phytochemical by GC-FID

The analysis of phytochemical was performed on an Agilent 6890 Gas chromatography equipped with a flame ionization detector according to the method of Buss and Butler (2010). A RESTEK 15 meter MXT-1 column (15m × 250um × 0.15um) was used. The injector temperature was 280^{o}C with splitless injection of 2µl of sample and a linear velocity of 30cms^{-1}, Helium 5.0par was the carrier gas with a flow rate of 40 mlmin^{-1}. The oven operated initially at 200^{o}C, it was heated to 330^{o}C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 320^{o}C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in part per million (ppm).

Results

The results of the GC-FID Analysis of the chemical constituents of the boiled water extracts of the leaves of *F. capensis, J. carnea, M. pruriens* and their combination is presented in Table 1. The result shows that the extract of *F. capensis* has the most abundant phytochemical, catechin, among all the phytochemicals present in the extracts. It is present at a concentration of 190.06ppm in the *F. capensis* extract while *J. carnea, M. pruriens* and their combination showed 0.57, 1.84 and 0.51ppm of catechin respectively. Second to catechin in abundance is Kaempferol which was also found to have its highest concentration in the *F. capensis* extract (58.97ppm) whereas *J. carnea, M. pruriens* and their combination showed 21.44, 35.87 and 0.83ppm respectively. The combination had the least amount of these two phytochemicals. The highest concentration of Apigenin was found in the *J. carnea* extract (30.05ppm) while the least amount was found in the combination. Also Sylimarin was only found in *J. carnea* extract while Revertrol was absent in *M. pruriens* extract but Present in others. Other phytochemicals that are present in varying concentrations in all the extracts includes: Quercetin, Luteolin, Artemetin, Retusin, Ellagic acid, Vanillic Acid, Naringenin, Apigenin, Isorhamnetin, Hesperidin, Maricetin, Epicatechin, Daidzein, Genistein, Apigenin, Lunamarin, Gallocatechin and Tangeretein

Discussion and Conclusion

From the results, it can be seen that catechin was predominat in the extract of *Ficus capensis* (190.06 ppm). Catechin is a flavan-3-ol, a type of secondary metabolite providing antioxidant roles in plants. Antioxidants are often used to protect cells and tissues and reverse oxidative damage (Imam *et al.,* 2017). Many bioactive plant-derived compounds have been shown to regulate both iron metabolism and redox state, possibly through interactive mechanisms. The catechin was also found in the extracts of *J. carnea, M. pruriens* and their combination at concentrations of 0.57, 1.84 and 0.51ppm respectively. The presence of these compounds at appreciable amount in these leaves extracts may be responsible for their reported biological activities such as antioxidant, antiinflamatory, anti anemic among others (Asogwa *et al*., 2020; Ezeigwe *et al*., 2021).

Table 1: GC-FID Analysis of the phytochemical composition of the boiled water extract of the leaves of *Justicia carnear, Ficus capensis, Mucuna pruriens* and their combination.

Phytochemicals (ppm)	*F. capensis*	*J. carnea*	*M. pruriens*	Combination
Kaempferol	58.97	21.44	35.87	0.83
Catechin	190.06	0.57	1.84	0.51
Quercetin	2.76	0.68	0.65	1.75
Luteolin	10.17	0.44	10.36	1.51
Artemetin	8.32	0.37	1.59	5.67
Retusin	11.46	23.65	0.40	0.47
Ellagic acid	3.74	1.28	0.59	2.81
Vanillic Acid	1.49	0.68	0.59	0.56
Naringenin	2.99	0.42	5.81	6.07
Apigenin	2.73	30.05	1.93	0.31
Isorhamnetin	12.79	0.14	0.51	1.68
Hesperidin	29.67	0.31	19.48	1.99
Maricetin	1.08	0.3	2.31	0.37
Epicatechin	6.05	11.32	2.31	7.85
Daidzein	0.22	1.25	0.39	0.88
Genistein	0.24	1.09	0.45	0.15
Apigenin	0.35	0.99	2.47	0.17
Lunamarin	25.66	0.90	1.29	0.48
Gallocatechin	25.11	0.85	0.13	0.39
Revertrol	28.79	0.73	-	1.69
Tangeretein	42.42	0.53	90.58	20.32
Silymarin	-	0.29	-	-

Note: - means the phytochemical was not detected.

Second to catechin in abundance is Kaempferol which was also found to have its highest concentration in the *F. capensis* extract (58.97ppm) whereas *J. carnea*, *M. pruriens* and their combination showed 21.44, 35.87 and 0.83ppm respectively. This could be due to dilution effect as the concentration of each of the extracts was redued by a factor of three in the combined extracts. This dilution may lead to reduction in toxicity in the case of toxic phytochemicals. Kaempferol is a flavonoid; flavonoids are regarded as the largest group of secondary plant metabolites (Imam *et al.*, 2017). There has been an increasing amount of research interest in the anti-carcinogenic potential of kaempferol, as a positive correlation between its consumption and reduced cancer incidence has been documented; this is in addition to existing epidemiological studies linking increased flavonoid consumption with reduced cancer incidence (Weng and Yen, 2012).

Flavonoids has been reported to possess antioxidant properties (Asogwa *et al.*, 2020). The combination had the least amount of these two phytochemicals (Catechin and kaempferol). Studies have shown that over consumption of Catechin can lead to hepatotoxicity (Abu *et al.*, 2005). Thus the use of the combined extract can lead to reduced toxicity. Traditional medicine often involves the combination of two or more extracts, oils, or infusions, based on the assumption that such mixtures will enhance therapeutic effects while minimizing adverse effects in comparison to conventional pharmaceuticals (Deciga-Campos *et al.*, 2021). The highest concentration of Apigenin was found in the *J. carnea* extract (30.05ppm) while the least amount was found in the combination. Viola *et al.* (1995) reported that overdose of apigenin can lead to sedation. Thuse, the combination of the extracts may have diluted the concentration of apigenin in the extracts and thus, could reduce its potential toxicity. It is known that apigenin can regulate intrinsic apoptotic pathways, changing mitochondrial membrane potential and causing the release of cytochrome C in the cytoplasm, which subsequently forms APFA, activates caspase 3, and turns on apoptosis (Seo *et al.*, 2017). Numerous plant metabolites, including alkaloids, saponins, flavonoids, and phenolic acids, have demonstrated the ability to influence fertility regulation (Udedi *et al.*, 2020) which is dependent on their concentration. Thus Herbal drugs or medicinal plants which have shown a wide range of biological activities and are used as medicine or food supplements to alleviate various ailments and disorder can also have deleterious effect if their dosage are not standardized. Combination of these herbs during preparation of herbal remedies may improve their efficacy and reduce their toxicity (Deciga-Campos *et al*., 2021).

Sylimarin was only found in *J. carnea* extract while Revertrol was absence in *M. pruriens* extract but Present in others. Research has shown that silymarin treatment significantly reduced serum iron, ferritin, serum hepcidin, and soluble transferrin receptor (sTfR), demonstrating the beneficial potential of silymarin as an iron chelating agent in reducing the serum ferritin and iron level in β-thalassemia (Cotoraci *et al.*, 2021). Other phytochemicals that are present in varying concentrations in all the extracts include: Quercetin, Luteolin, Artemetin, Retusin, Ellagic acid, Vanillic Acid, Naringenin, Apigenin, Isorhamnetin, Hesperidin, Maricetin, Epicatechin, Daidzein, Genistein, Apigenin, Lunamarin, Gallocatechin and Tangeretin. Tangeretin was found in high amount in *M. pruriens* leaf extract and it is a

key member of flavonoids that has different favourable biological activities such as antioxidant, anti-inflammatory, anti-tumor, hepatoprotective and neuroprotective effects (Ashrafizaheh *et al.*, 2020). Some of these phytochemicals act directly to induce the resolution of anemia, and others act pleiotropically through their antioxidant activity, by increasing oxidative stress resistance or by triggering cellular mechanisms, such as autophagy, or, for example, by targeting inflammation in the elderly population and subsequently reducing the anemia associated with chronic inflammation (Varoni *et al*., 2015).

Conclusion

In conclusion, the boiled water extracts of the leaves of *Justicia carnea*, *Ficus capensis* and *Mucuna pruriens* are reach in bioactive compounds which may confer them some medicinal properties. Their combination led to changes in the concentration of the phytochemicals present in the individual samples. This observed variation suggest that there may be herb to herb interaction. This interaction may lead to incread in potency of the herbal remedy and decrease in toxicity as observed in the reduction in concentration of some of the phytochemicals.

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