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PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *TETRAPLEURA TETRAPTERA* SEED EXTRACTS

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ABSTRACT

Tetrapleura tetraptera, also known commonly as Prekese or Aidan fruit in Ghana and Nigeria, respectively, is noted for its use in herbal preparations. The fruit in particular is employed as an ingredient in traditional medicine and as a spice across West-Africa. The seed, which forms part of the fruit, is discarded together with the spent fruit. This study aimed to evaluate the phytochemical composition as well as the biological activity of the hexane and ethanol extracts of the prekese seed. Our investigation revealed that the extracts contained valuable secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and glycosides. All extracts exhibited mild to good levels of antimicrobial activity against the tested microorganisms. The ethanol extract had a better biological activity compared to the hexane extract. The biological activities of both the hexane and ethanol extracts were lower compared to those of ciprofloxacin and ketoconazole.

Keywords: *Tetrapleura tetraptera*, prekese, aidan fruit, phytochemicals, biological activities

Introduction

Traditional medicine continues to be a major part of the health delivery system in Ghana (Barimah, 2016; Agyei-baffour *et al*., 2017; Boadu & Asase, 2017). In 2005, the National Health Policy was amended to pave way for the integration of herbal medicine into mainstream healthcare (Ministry of Health, 2005). Consequently, in 2012, herbal medicine practice was formally integrated into the main healthcare delivery system in Ghana with a pilot of about 18 government facilities nationwide (Asare, 2015). Herbal medicine registration in Ghana is conducted by the Food and Drugs Authority (FDA) of Ghana for the treatment of different diseases (FDA, 2013). In all these approved herbal medicines, various plant parts are reported as the major ingredient. In Ghana and across the West-Africa subregion, *Tetrapleura tetraptera* (Prekese) is found in most of these registered herbal medicines. *Tetrapleura tetraptera* (Prekese) is a perennial plant with dark green leaves and thick, woody base and spreading branches. The plant has a wide natural distribution over a large part of tropical Africa, especially in the rain forest belt of West, Central, and East Africa. The fruit consists of a fleshy pulp with some small, brownish - black seeds. The fruits are green when tender and dark red-brown when fully ripe. The fruits are about 22 cm – 27 cm long, pods 4 – 5 cm wide, and have four longitudinal wing-like rather fleshy ridges about 2 cm wide of which two are hard and woody (Aladesanmi, 2006).

The utilisation of prekese leaves, fruit, and stem bark in traditional medicine does not end at the commercial production of herbal medicine. Not only that but also the Prekese fruit is used in various households as a spice (Abugri & Pritchet, 2012). Prekese has been utilised in traditional medicine for the treatment of several illnesses. The fruit has been found to possess antimicrobial, molluscidal, insecticidal, antiproliferative, anticancer, anticonvulsant activities (Aikins *et al.,* 2021; Adusei *et al*., 2021; Saliu *et al*., 2021; Lin *et al*., 2019; Mbaveng *et al.,* 2021). The chemical composition and biological activity of the prekese fruit has also been investigated (Aladesanmi, 2006; Abugri & Pritchet, 2012). However, the seed of the fruit has received minimum attention. At the Council for Scientific and Industrial Research - Forestry Research Institute of Ghana (CSIR-FORIG), the prekese fruit is processed into a food supplement called the Prekese Syrup. Large quantities of the Prekese fruit waste are generated after processing. This waste includes the Prekese seeds. The seeds of Prekese are believed to be utilised by the rural folk for the treatment of poultry diseases. The seeds are thought to possess some biological activity. Korang *et al*., in 2019 reported the effectiveness of the ethanol extract of Prekese seed as a fungus agent. Aside the extracts from the fruit pulp, some antimicrobial activity of the Prekese fruit can be speculated to be as a result of the phytochemicals in the seed extract rather than from the fruit extract. This is because most investigations on the biological activity of the fruit failed to separate the seed extract from the fruit extract. This makes the study very interesting since this will be the first time the antimicrobial activity of the seed extract alone has been investigated separately.

Experimental

Sample collection

The Prekese seed hexane extract (PSHE) and Prekese seed ethanol extract (PSEE) were extracted from prekese seeds obtained from National Tree Seed Centre (NTSC).

The extraction of PSHE and PSEE was performed using hexane and ethanol successively on the same sample. This process is to make sure the PSHE contains mainly nonpolar extractives while the PSEE contains mostly polar extracts.

Phytochemical screening

The phytochemical screening of the extracts was done using the standard procedure as described by Edeoga *et al.* (2005) and (Sofowora, 1992).

Minimum Inhibitory Concentration (MIC) determination

The Minimum Inhibitory Concentration (MIC) values of the extracts positive control, Ciprofloxacin, and Ketoconazole against bacterial and fungal isolates were determined. The broth dilution method was used to analyse the Minimum Inhibitory Concentration (MIC) of the extracts in 96 – well plates. Two-fold dilutions of 200 mg/mL to 1.5625 mg/mL concentrations were prepared with sterile double strength nutrient broth. One hundred and ninety microlitres (190 µL) of the diluted extract concentrations were dispensed into plates. The standard drugs, ciprofloxacin and ketoconazole were prepared in the same way as the extracts from 10 µg/mL to 0.078125 µg/mL and 1000 µg/mL to 7.5625 µg/mL respectively. Twenty microlitres (20 µL) suspensions of 0.5 McFarland standard test

microbes were distributed in each well to make a final volume of 200 µL. The plates were cultured in an incubator at 37^{O}C for 24 hours, after which 20 µL of 1.25 mg/mL tetrazolium bromide solution was added. Wells that developed purple colour after addition of MTT indicated the growth of test microbes and those that retained colour yellow was an indication of the inhibitory activity of the extract against the test microbes (Andrews, 2001).

Results and discussion

The results of the qualitative phytochemical tests are shown in Table 1. Most of the phytochemicals were present in low quantities. Sterols and coumarins were not detected in all extracts. Glycoside was the only phytochemical present in all extracts. Aside glucosides, the phytochemicals detected in PSEE were tannins and flavonoids and in PSHE were alkaloids and saponins. The presence of alkaloids and saponins in PSHE but absent in PSEE indicated the solubility of these secondary metabolites in hexane. However, the tannins and flavonoids present in the PSEE were not readily soluble in hexane. Igwe and Akabuike, 2016, reported the presence of flavonoids, alkaloids, tannins, saponins, and phenols in prekese extracts.

TABLE 1

Phytochemical screening results

Test Compounds	PSEE	PSHE
Alkaloids	- -	- +
Tannins	- +	- -
Glycosides	- +	- +
Saponins	- -	- +
Flavonoid	- +	- -
Coumarins	- -	- -
Sterols	- -	- -

Key: Absence (- -), Low present (-+), Present (+ +)

The phytochemicals detected in the extracts were alkaloids, flavonoids, tannins, saponins, coumarins, and glycosides and they possess medicinal properties. The biological activities of the plant extracts were attributed to the occurrence of these natural compounds (Ojo *et al.,* 2012). Flavonoids are natural substances with variable polyphenolic structure and have been reported to possess antiallergic, antioxidant, and anticancer properties (Panche *et al*., 2016). Alkaloids are an assembly of naturally occurring chemical compound composites which typically comprise of basic nitrogen atoms (Manske & Holmes, 2014) with pharmacological properties such as hypotensive, anticonvulsant, anti-protozoal, antimicrobial and antimalarial activities (Dey & Bhakta, 2012; Karuna *et al*., 2018a; Kundu *et al*., 2019; Gorain *et al*., 2014). Tannins are important natural bioactive compounds found in different forms. They are reported to possess antitumor, antiviral, and antibacterial activities (Das *et al*., 2020). Saponins possess antioxidant, anticancer, anti-inflammatory, hyperglycaemic, and antifungal properties (Dong *et al*., 2020). The secondary metabolites will confer on these extracts some biological activity.

The extracts were tested against 10 bacteria and 1 fungus. The activities of the extracts are reported in Tables 2 and 3. The extracts showed some biological activity towards the test organisms. These observations against both gram-positive and gram – negative organisms indicated the broad-spectrum activities of these plant extracts. The concentration of the extracts was varied from 1.56 mg/ml to 200 mg/ml. The MIC, which is the lowest concentration of a chemical, usually a drug, prevents visible growth of a bacterium or bacteria was measured. The MIC varies for each extract against a particular microorganism.

TABLE 2

Minimum Inhibitory Concentration (g/ml) of Prekese Seed Hexane (PSHE).

Test organisms	Concentrations (mg/ml)							
	200	100	50	25	12.5	6.25	3.13	1.56
E. coli	-	-	-	-	-	-	+	+
K. pneumoniae	-	-	-	-	+	+	+	+
B. subtilis	-	-	-	-	-	+	+	+
E. faecalis	-	-	-	-	+	+	+	+
P. aeruginosa	-	-	-	-	-	+	+	+
P. mirabilis	-	-	-	-	+	+	+	+
S. typhi	-	-	-	+	+	+	+	+
S. aureus	-	-	-	-	-	+	+	+
MRSA	-	-	-	+	+	+	+	+
S. pyogene	-	-	-	+	+	+	+	+
C. albicans	-	-	-	-	+	+	+	+

Key: - Means no growth, + Means growth

TABLE 3

Minimum Inhibitory Concentration (g/ml) of Prekese Seed Ethanol (PSEE)

Test organisms	***Concentrations (mg/ml)***							
	200	100	50	25	12.5	6.25	3.13	1.56
E. coli	-	-	-	-	-	+	+	+
K. pneumoniae	-	-	-	-	-	+	+	+
B. subtilis	-	-	-	-	+	+	+	+
E. faecalis	-	-	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	-	+	+	+
P. mirabilis	-	-	-	-	-	+	+	+
S. typhi	-	-	-	-	-	-	+	+
S. aureus	-	-	-	-	-	+	+	+
MRSA	-	-	-	+	+	+	+	+
S. pyogene	-	-	-	-	+	+	+	+
C. albicans	-	-	-	-	-	-	+	+

Key: - Means no growth, + Means growth

TABLE 4

Minimum Inhibitory Concentration (MIC) of Ciprofloxacin against the test organisms

Test organisms	Concentrations (µg/ml)							
	10	5	2.5	1.25	0.625	0.3125	0.156	0.0781
E. coli	-	-	-	+	+	+	+	+
K. pneumoniae	-	-	+	+	+	+	+	+
B. subtilis	-	-	-	+	+	+	+	+
E. faecalis	-	-	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+
P. mirabilis	-	-	-	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+
S. aureus	-	-	-	+	+	+	+	+
MRSA	-	-	+	+	+	+	+	+
S. pyogene	-	-	-	+	+	+	+	+

Key: - Means no growth, + Means growth

TABLE 5

Minimum Inhibitory Concentration (MIC) of Ketoconazole against C. albicans

Test organisms	Concentrations (µg/ml)							
	1000	500	250	1.25	0.63	0.31	0.16	0.08
C. albicans	-	-	-	+	+	+	+	+

Key: - Means no growth, + Means growth

It was observed that the activity of the extracts increased with increasing concentration, which was consistent with expectation. The antimicrobial activity of the most effective antibacteria agents increases linearly with concentration. This implied that the higher the concentration of the extract, the greater the zone of inhibition.

The bioactivities of the extracts were compared with the activity of a known antibiotic and antifungal drug as standards. The MIC's of the standards Ciprofloxacin and Ketoconazole were reported in Tables 4 and 5, respectively. The MIC of the three extracts were very high compared to the standards. This indicated that the effectiveness of the extracts was very low compared to the standards.

The comparison of the two (2) extracts is essential to determine which solvent to utilize for subsequent investigation. Generally, for each microorganism, the PSEE was very effective compared to the PSHE. It was only the B. subtilis that the activity of PSHE was better than PSEE. This might be due to the mode of attack or receptors on the bacteria.

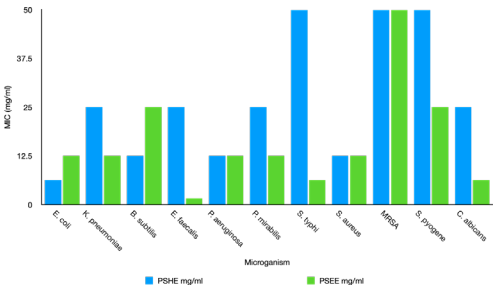

Fig 1: Comparison of MIC of the two extracts

Conclusion

Five phytochemicals (alkaloids, flavonoids, tannins, saponins, and glycosides) were detected in the prekese seed extracts. The extracts exhibited a mild to good level of antimicrobial activity against the tested microorganisms. The ethanol extract was very effective compared to the hexane extract. The extract activity was lower compared to ciprofloxacin and ketoconazole. However, in traditional medicine the extracts can be essential due to the high dosage administered.

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