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Assessment of the Essential Oil and Antibacterial Activities of the Seeds of *Psophorcapus tetragonolobus* "Winged Bean Seeds"

Abdulmalik Fatima A.*[1], Shoge Mansurat,[1], Tamasi Anas Ali,[1]
Adegboyega Taofeek Tope,[2] Ozioko Eucharia Ngozi,[2] Aliyu Mutiu. O[1]

[1]Department of Chemistry, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria.
[2]Biology Unit, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria.

Corresponding Author's Email: moshachemist@yahooo.com
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Abstract

Bacteria are a major causative agent for different types of diseases. Therefore, it is necessary to get rid of these bacteria using naturally derived products. In order to find a better natural product as a remedy to combat bacteria, this research investigated the application of essential oil extracted from *P. tetragonolobus* seed through soxhlet extraction process. The yield of oil obtained from 100 g of the pulverized sample was 3 g. Subsequently the antimicrobial activity of the winged bean seed oil was assessed. The *in vitro* analysis was done against (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococus aureus* and *Salmonella typhi*) using standardized procedures. Results demonstrated notable zones of inhibition, which indicates significant antimicrobial efficacy. Pseudomonas aeruginosa strain was the most susceptible to the oil extract's antibacterial activity, which was surpassed by Salmonella typhi, Escherichia coli, and Staphylococcus aureus. The control, ciprofloxacin, had a zone of inhibition of 60mm, thereby, demonstrating a notable level of activity. However, the extract's zones of inhibition had diameters of 40, 29, and 35 mm, placing Pseudomonas aeruginosa in the sensitive organism category. All of these results that extract from the seed oil of this plant can be used as an antibiotic in the treatment of various ailments, according to claim by the local herbal medical practitioners. The GC-MS analysis of the oil extract also reveals the presence of some phytocompounds including Benzenedicarboxylic acid, bis (2-ethyhexyl) ester, Gamma-sitosterol, Octacosanol, Octadienoic acid, Gamma-tocopherol and others. Furthermore, the Fourier Transformed Infrared spectroscopy (FTIR) provided insight into the functional groups present in the oil, confirming the presence of OH group which is present in most of the compounds including stigmasterol, docosanoic acid, aromatic amines, carboxylic acids among others. The study shows the oil's potential as a natural antibacterial agent in addition to providing a thorough chemical analysis of it.

Keywords: Seed Oil, Bacteria, Antibacterial activity, Antibiotic, Zone of Inhibition, Soxhlet extraction, TLC analysis, GC-MS Analysis, FTIR Analysis.

1.0 Introduction

Pathogenic bacteria are a significant source of various infectious diseases in humans, animals and plants. They involve a mechanism to evade the host's immune system and exploit their environment to multiply and cause harm. Antibiotics are effective in treating a wide range of

bacterial infections, from common respiratory infections and more severe conditions like urinary tract infections and pneumonia (Smith *et al*., 2020). Despite their significance, the continuous use of antibiotics initiates antibiotic resistance which poses a global health threat, also, overuse or misuse of these antibiotics can lead to the development of resistant bacterial strains, reducing the effectiveness of these critical medications over time (Miller and Green, 2019). Natural products often harbor a diverse array of bioactive compounds that may offer novel antibacterial agents (Jones and Brown, 2018). This approach aligns with increasing demand for sustainable and eco-friendly alternatives in pharmaceutical research. Studying natural sources of antibacterial compounds such as seed oil is crucial due to their potential therapeutic applications and literature considerations (Smith *et al*.,2020).

Medicinal plants have been used in healthcare since ancient times. The emphasis on the use of medicinal plants had been placed on the treatment rather than prevention of diseases. The literature has reported on studies on the use of medicinal plants and their components in the prevention of diseases. World Health Organization (WHO, 2020).

Legumes are members of the family Fabaceae or Leguminosae and include economically important grain legumes, oilseed crops, forage crops, shrubs, and tropical or subtropical trees. Legumes are a rich source of quality protein and also enrich the soil by producing their own nitrogen in symbiosis with nitrogen-fixing bacteria (Singh *et al.,* 2017).

The prevalence of diabetes mellitus (DM) is rapidly increasing worldwide as a result of population growth, ageing, urbanization and lifestyle changes, resulting obesity and physical inactivity. The consumption of legumes has been associated with a lower risk of developing several chronic diseases, mainly obesity and type 2 diabetes (Schröder, 2017). Lunasin is found in Soybean and also detected in wheat and barley and classified as having antimicrobial properties, blood pressure-lowering effects, cholesterol-lowering ability, antithrombotic impacts and antioxidant activities (Mejia & Dia, 2010). Legumes, particularly beans, contain bioactive components like phenolic compounds (flavonoids, tannins, and anthocyanin), protease inhibitors, phytic acid, and saponins. These components have helped in the inhibition of colon rectal cancer (Harland & Morris, 2022).

Winged bean (*Psophocarpus tetragonolobus*) also locally called "*kakaaki"* in Hausa, "*oogun alakiisa"* in Yoruba and "agwa" in Igbo is an underutilized legume widely cultivated in Asia and Africa. All parts of the plant, from the seeds and immature pods to the leaves, flowers and tuberous roots are edible. It is comparable to soybean in terms of composition and nutritional value of the seed, because both contain a similar proportion of protein, oil, minerals, vitamins and essential amino acids (Amoo *et al*., 2016). More recently, an increased understanding of nutritional, anti-nutritional and chemical properties of this legume has been established (Lampinen *et al.,* 2017). Traditionally, the winged bean is used to treat diabetes, cancer, infection, eye and migraine diseases, muscle weakness, and asthma (Singh et al. 2019). Previously, several studies have reported pharmacological activities of winged beans, such as an antioxidant (Singh *et al.,* 2019). *P. tetragonolobus* protein is safe in terms of genotoxicity, cytotoxicity and hence has the potential for use as a key component in pharmaceutical and food industries (Chen *et al.,* 2015). The fatty acid composition of *P. tetragonolobus* is compared to *Arachis hypogaea* (groundnut) and was found to have higher amounts of behenic and paranaric acid which are good antioxidant agents (Mohanty *et al.,* 2014). Compared to soy bean and corn oil, *P. tetragonolobus* contains long chain fatty acid and fairly small amount of polyunsaturated fatty acid which is favourable for oil stability against the fungi *C. albicans* (Mohanty, 2014). These findings indicate that *P. tetragonolobus* is a good source of oil with high quality having diverse economic significance (Singh *et al*., 2023).

This research aimed to investigate the In-vitro antibacterial activity studies from the oil from the seeds of *Psophorcapus tetragonolobus* against various pathogenic bacteria through the process of extraction of oil from the seed of *P. tetragonolobus* using soxhlet extraction method, conducting the antimicrobial screening of the oil extract to confirm the level of potency against various bacterial strains, conduct Thin Layer Chromatography (TLC) to determine the components present in the oil extract. Thus, carry out comparative analysis of the potency of the isolated compounds against a standard antimicrobial drug and conduct spectroscopic analyses of the oil extract using FT-IR and GC-MS**.**

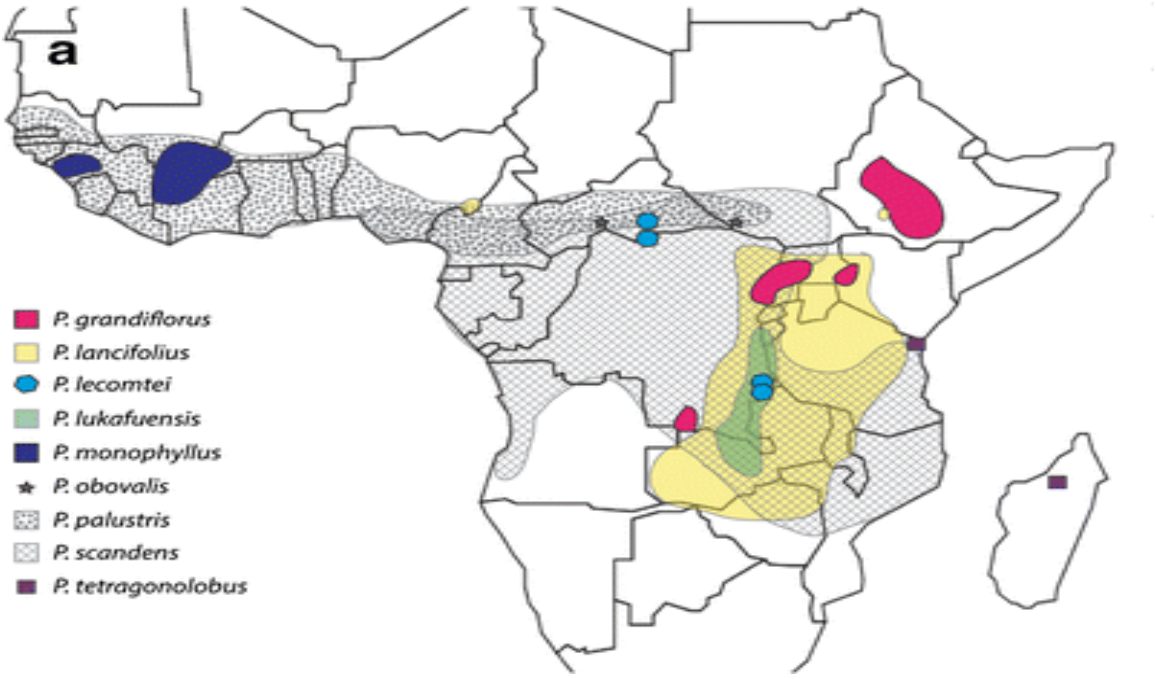

Figure 1: Distribution of *Psophocarpus tetragonolobus* in Africa

2.0 Materials and Methods

Sampling and Preparation of Plant material

The seeds of *Psophocarpus tetragonolobus* were collected from A.B.U. Zaria Biological Garden in Sabon gari Local Government Area of Kaduna State, Nigeria. The sample were identified and authenticated by Dr. Yahaya in the Herbarium Section, Department of Biological Sciences, Nigerian Defense Academy (NDA), Kaduna state, Nigeria, and was assigned the voucher number (NDA/BioH/2023/15). The seeds were properly prepared by removing the stones and dirt. The seeds were air dried in the shade for 7 days, pulverised into fine powder using a vibrating cup mill apparatus, and then kept in an airtight jar for use.

Extraction of the Seed Oil

According to (Sule *et al.,* 2020) method of extraction with slight modification was used,50g of powdered sample, and was poured into a cone-shaped filter paper, tied using a white thread, and then placed into a thimble (semi-permeable membrane) which was later placed into the sample chamber of the soxhlet apparatus, 250ml of n-hexane was measured into a round bottom flask of the apparatus and a sample-solvent ratio of (1:5w/v) was used, where the condenser was fixed tightly at the end of the extractor. The whole set up was heated in the heating mantle at a temperature of 60^{o}C until the solvent reached its boiling point and the vapour raised, and dripped onto the sample. This process continued in circles, moving the oil extract back to the flask.

The extraction process was carried out for 2 days until an optimum yield of oil extract was achieved.

The weight of the oil extract was taken after all the solvent has evaporated and was used to calculate the percentage yield using equation (1) below. The oil quality parameters were obtained.

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of extract(g)}}{\text{Weight of sample(g)}} X\ 100 \qquad (1)$$

Antibacterial Assay

Test Organisms

The bacterial organisms that were used for the anti-bacterial assay of this research work are*; Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella enterica* which were obtained from the stock culture of Microbiology Laboratory of National Agency for Foods and Drugs Administration and Control (NAFDAC), Kaduna state, Nigeria in which they were grown on Trypton Soya Agar (TSA).

Microbial Growth Media Preparation

The steps in preparing trypton soya agar (TSA)

65g of solid medium agar was added in 1L of distilled water and was well combined by vigorous shaking. followed by continuous stirring while heating until fully dissolved. The solution was cooled in a water bath to 45°C after being autoclaved for 15 mins at 121°C. Then the agar was poured into sterile petri dish on a flat horizontal surface, ensuring that each 90mm plate had a uniform depth of about 25ml of liquid agar. The plates were then left to solidify and stored at 4°C until ready for use. All the test organisms; *Salmonella typhi, Psedomonas aeruginosa, Staphylococcus aureus and Escherichia coli* were cultured on the prepared TSA.

Determination of Anti-Bacterial Activity

The anti-bacterial activity of *P. tetagonolobus* seed oil was determined using a method adopted by (Ajiboye and Oyejobi 2017). The bacterial isolates were grown in nutrient broth for 18 hours at 35ºC. The inoculum load was adjusted to $1x10^{-3}$cfu /ml via serial dilution method prior to use. The antibacterial activity of the oil extract against the test organism was screenedusing the agar well diffusion method. An inoculum suspension was swabbed uniformly to solidified 20ml tryptone soya agar (TSA) and the inoculation was allowed to dry for 5 minutes. Holes of 5mm in diameter were made in the seeded agar using sterile cork-borer. Aliquot of 50µl from the oil extract was added into each well on the seeded medium and allowed to stand on the bench for 1 hour for proper diffusion and thereafter, incubated at 37ºC for 24 hours. The resulting inhibition zones were measured in millimeters(mm). The positive control employed is ciprofloxacin antibiotic. These studies were performed in triplicates i.e. oil extract only, positive control (ciprofloxacin tablet), and the extract + positive control. For the oil extract with organism, 5mm hole in diameter was made on the TSA medium containing each organism and then 5µl of oil was added. For positive control only, ciprofloxacin anti-biotic was placed on each organism medium and finally for the oil extract + positive control, three 5mm holes were made o? 4 media prepared for each organism, the three holes were labelled 0.5, 1.0, 1.5 respectively. For the 0.5 hole, 0.5ml of the oil and 0.5ml of the control solution was added, for 0.1 hole, 1ml of the oil and 1ml of the control solution was added, finally, for the 1.5-hole, 1.5 ml of oil and 1.5ml of the control solution was added.

The diameters of the clear zones of inhibition were measured and the results were recorded to the nearest (mm). The diameter of the zone is related to the susceptibility of the isolate and the diffusion rate of the oil extract through agar medium.

Gas Chromatography-Mass spectrometry (GC-MS) Analysis

GC-MS analysis of *Psophorcapus tetragonolobus*, seed oil extract was carried out by adopting the method of (VasudhaUdupa *et al*., 2021). Gas chromatography (GC) (Thermos Scientific Trace1310) and Triple Quad mass spectrometry were used to determine the chemical composition of the oil extract. With DB 5MS (30cm length, 0.250mm ID, spectrometer (MS) (Thermos scientific TSQ 8000) and helium as the carrier gas. A single 1µl was introduced into the 250ºC column. A column temperature initiated the GC program at 40ºC for 2 minutes and increased to 240ºC at $5ºCm^{-1}$ rate. Furthermore, the temperature was increased to 300ºC at a scan range of 50-600 Da. The total run time was 47 mins. The data was analyzed by the software "Calibur 4.0" and the chromatogram obtained were analyzed and identified by matching their spectral fragmentation patterns with a database deposited at the National Institutes of Standards and Technology Mass Spectral Database (NIST 2.2) library.

3.0 Results and Discussion

Percentage Yield of Extract from 100g of Pulverized Seed Sample

The mass of the oil extract obtained from the extraction and percentage yield of 100g of pulverized sample is shown in below in Table 1.

Table 1. Percentage Yield of Extract

Solvent for extraction	Yield (g)	Percentage Yield(%)
n-hexane	**3**	**3**

Antibacterial Activity Test of Oil Extract

The result of antibacterial activity test of the oil extract against the clinical isolates of *E. coli, P. aeruginosa, S. aereus, and Salmonella typhi* is given below:

Table 2: Drug and extract zones of inhibition (mm) Concentration (%)

S/N	*S. aureus*					
1			0.5	1.0	1.5	Drug (0.5)
	Extract		40	29	35	-
	Ciprofloxacin		-	-	-	65
2	*P. aeruginosa*					
			0.5	1.0	1.5	Drug (0.5)
	Extract		40	29	35	
	Ciprofloxacin		-	-	-	
3	*S. typhi*					
			0.5	1.0	1.5	Drug (0.5)
	Extract		30	35	30	
	Ciprofloxacin		-	-	-	57
	E. coli					
4			0.5	1.0	1.5	Drug (0.5)
	Extract		25	20	30	
	Ciprofloxacin		-	-	-	55

Table 3: TLC R_f of Extract

Extract	No. of Spots	R_f Values
***P.tetragonolobus* seed oil**	**3**	**0.125, 0.25, 0.50**

Table 4. Biological Activity of Some of the Compounds Identified by GC-MS

S/N	Compound Name	Biological Activity
1	**Beta-carotene**	Antioxidant, Reduces the risk of cardiovascular diseases (Reygaert, 2019)
2	**Squalene**	Anti-inflammatory, antioxidant
3	**Gamma-sitosterol**	Anti-cancer,Anti-inflammatory, antioxidant (Fajardo *et al*., 2018)
4	**Cyclononasiloxane**	Antioxidant, antioxidant (Rizkalla *et al*., 2012)
5	**Kaur-16-ene**	Antioxidant, antimicrobial (Kumar *et al*., 2015)

According to this research, there was less oil than expected, the yield of the oil extracts can vary significantly depending on the quality and composition of the plant material used. Factors such as species, growing conditions, age of the plant, and harvesting methods can all influence the oil content and yield (Guenther, 1952). The choice of extraction method plays a crucial role in determining the yield of oil extracts. Different techniques like solvent extraction, cold pressing, or steam distillation can yield varying amounts of oil due to differences in efficiency and selectivity for certain compounds (Lutterodt *et al*., 2011). Some plants may contain oil in specialized structures (e.g., oil glands) that are not easily accessible or are difficult to extract efficiently. The complexity of the plant's chemical composition and the structure of its tissues can hinder the extraction process and reduce overall yield (Croteau *et al*., 2000). During the extraction process, losses can occur due to degradation of heat-sensitive compounds, volatilization of oils, or adherence of oil to plant residues or extraction equipment (Croteau *et al*., 2000). Oil content in plants can vary throughout the year due to seasonal changes in environmental conditions and plant physiology. This variability can affect the overall yield of oil extracts (Guenther, 1952). Achieving high purity and quality of oil extracts often requires additional purification steps, which can further reduce the overall yield (Lutterodt *et al*., 2011). The oil extracted by soxhlet extraction method from *P. tetragonolobus* seeds had high antibacterial activities (using agar well diffusion method) against the screened pathogenic bacterial strains (*P. aeruginosa, E. coli, S. aureus and Salmonella typhi*). Thus, investigations on the antimicrobial activity of various oil extracts against different pathogens have been performed worldwide (Dorman and Deans 2014). These results have significance because they provide information about this project. The oil of *P. tetragonolobus* seed reacted positively to the tested microbial strains ranging from 29-60mm. Under similar conditions of extraction and experimentation, *Opuntiaficus indica* oil was less efficient than *P. tetragonolobus* oil as the zone of inhibition was only clearly visible against *Salmonella typhi.* (Khémiri *et al.,* 2019). However, no activity has been detected against *E.coli*, *Staphylococcus aureus* and *P. aeruginosa* based on diameter of inhibition zone measurement .(Khémiri *et al.,* 2019). A recent study showed that methanolic and aqueous extracts from the seeds of this species had antibacterial activities against *Escherichia coli* and *Acinetobacter baumanii* with inhibition diameters of 3mm and 5mm, respectively, while based on MICs, the same extracts exhibited antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Abdel *et al.,* 2018).

The most effective, is the inhibition of ciprofloxacin antibiotic with a diameter of 60mm. Others showed significant efficacy with inhibition diametres of 29, 35 and 40. On the other hand, the zone of inhibition diametres of the seed oil extracts were 40, 29 and 35, classifying *P. aeruginosa* in the group of sensitive organisms. (Rota *et al.,* 2018). The differences in microorganism sensitivity to the oil *P. tetragonolobus* seed was due to the quantity and quality of bioactive molecules, the nature of the cell

wall and cell's enzymatic system's strength, which controls metabolism. The in-vitro antimicrobial activity of the seed oil against *P. aeruginosa* can be attributed to the combination of different components present in the oil (Bertelle *et al*., 2018). (Singh *et al.*, 2019) also reported that winged bean is a natural source of an antibiotic. Several studies outlined that antibacterial activities of plant extracts are due to their oleic acid content which cause cell membrane disruption (Jaberian *et al., 2013*). *P. tetragonolobus* oil may contribute to inactivate bacterial growth.

Spectroscopic Analyses (FT-IR and GC-MS)

Table 7 show the GCMS analysis of the bioactive phytocompounds present in the oil extract of the seeds of. *P. tetragonolobus*. On comparison of the mass spectra of the constituent with the NIST14s library, the GCMS spectrum revealed that the oil extract contains up to 34 phytocompounds which include; steroids, carboxylic acids, esthers among others. The active principles with their retention time (RT), molecular formula, molecular weight and concentration (%) of that 28 phytoconstituents present in *P. tetragonolobus seed oil* are presented above. The spectrum profile of GC-MS which confirmed the presence of 34 major compounds are shown in Table 7 above. The presence of two (2) absorption bands (stretching vibrations) at around 2800-2912.6cm^{-1} which fall within the range of 2800-3000cm^{-1} signifies the presence of CH_3, CH_2 and CH. Such groups are observed in most of the structures from the GC-MS results like Kaur-16-ene, 9,12-Octadecadienoic acid, 9-Octadienoic acid e. t. c. Absorption at 3480.9cm^{-1} confirms the presence of OH group. OH groups are seen in most of the compounds including docosanoic acid, stigmasterol, 1-decanol, -hexyl, gamma sitosterol, octadienoic acid among others.Characteristic absorption of C=O group is observed at 1750.4cm^{-1} which would be due to aldehydes like 2-octenal, 2-decanal, carboxylic acids like n-hexadecanoic acid, oleic acid, esters like; ethylester, linoleic acid, e. t. c. Another absorption confirming ester group is at 1054.4cm^{-1}, signifying the presence of C-O group. Other absorptions are observed at 1156.6 cm^{-1} for -C-C-C- and at 2366cm^{-1} for C-H.

4.0 Conclusion

The oil of of *P. tetragonolobus* seed which was extracted by soxhlet extraction which was used to perform the antibacterial assay against four bacteria: *P. aeruginosa, E. coli. Salmonella typhi* and *Staphylococcus aureus.* The antimicrobial assay revealed a pronounced inhibitory effect of the oil extract on the growth of all tested bacterial strains where the zone of inhibition was directly proportional to the concentration of the extract. This suggests a broad-spectrum antimicrobial activity.

Underscoring the extract's potential as a versatile antibacterial infection. The inhibitory zones observed in wee diffusion assay indicates the extract's ability to combact pathogenic bacteria.

The subsequent GC-MS analysis provided valuable insight into the chemical composition of the oil extract where it revealed the presence of 34 major compounds. Summarily, the combination of antimicrobial activity of this oil as a potent source of bioactive compounds with notable antimicrobial which can be employed in utilizing and preparing drug.

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