

ORIGINAL RESEARCH ARTICLE

Quantitative Phytochemical Analysis and Antimicrobial Activity of *Ricinus communis* Linn. Seed oilMuhammad F. E.^{1*}  and Daniyan S. Y.¹¹Department of Microbiology, School of Life Sciences, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.

ABSTRACT

Ricinus communis (*R. communis*) is a plant in the spurge family that has been traditionally used to treat numerous ailments. The study aims to evaluate the phytochemicals present in *R. communis* seed oil and its antimicrobial efficacy. The phytochemicals were quantified using standard procedures, and antimicrobial activity was carried out using broth dilution method at -100, 50, 25, and 12.5 mg/ml of the seed oil against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Aspergillus niger*, and *Aspergillus flavus*. Alkaloids, flavonoids, phenols, saponins, and tannins were detected with varying concentrations. The highest MIC value, 100 mg/ml, was recorded against fungal isolates, while the lowest, 12.5 mg/ml, was recorded against *S. aureus*. Similarly, the highest MLC was recorded against the fungal isolates, and the lowest value of 50 mg/ml was recorded against all the bacterial species. The seed oil contained an appreciable amount of phytochemicals and exhibited antimicrobial activity against the tested isolates.

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INTRODUCTION

Medicinal plants are a rich reservoir of a vast array of active components produced in response to environmental stress and threats faced by these plants, acting as defensive tools for plant species and collectively termed Phytochemicals (Bose *et al.*, 2021; Nusrat *et al.*, 2021). They are naturally synthesized in the bark, leaves, stem, root, flower, fruits, and seeds of the plants, and their synthesis is time, tissue, and organ specific (Abdulrasheed *et al.*, 2015; Pradeepa *et al.*, 2016). These phytochemicals include phenolics, diterpenes, triterpenes, flavonoids, phenolic acids, sterols, and sphingolipids, among others that have significant therapeutic applications like antiviral, anticancer, analgesic, antitubercular, antiproliferative, antimicrobial and antioxidant potentials (Prasanta *et al.*, 2020; Nusrat *et al.*, 2021). Medicinal plants are extensively used in traditional medicine systems in many parts of the world, and their usage has been passed on from one generation to the other (Bose *et al.*, 2021). A common practice in Africa is the use of plant parts either as extract, infusion, decoction, tinctures, capsules, or seed oil for the prevention and treatment of various types of ailments such as cough, fever, gastro-intestinal disorder, liver and kidney diseases amongst others (Emejulu *et al.*, 2017). The oils of medicinal plants have been used to cure various ailments since men learned the art of their extraction (Momoh *et al.*, 2012). They are intriguing natural items that play a significant role in traditional pharmacopeia as a

feasible source for treating and managing various ailments (Arianne *et al.*, 2012). Some of these oils are beneficial in the cosmetics, nutraceuticals, and pharmaceutical industries and can suppress a number of plant diseases, human infections, and insects (Dada *et al.*, 2014).

Ricinus communis (*R. communis*) is a species of plant in the spurge family, *Euphorbiaceae*, which is one of the largest angiosperm families, with over 7,800 species distributed in roughly 300 genera and subfamilies in tropical and subtropical environments (Temesgen 2016; Hanan *et al.*, 2018). According to Ahmad *et al.* (2016), the name *Ricinus communis* is derived from two Latin words: '*ricinus*', which is the Latin name for the Mediterranean sheep tick (*Ixodes ricinus*), to which the castor plant seed has similarities, and '*communis*' which means common. Although called a "castor bean plant," it is not a true bean (Muhammad *et al.*, 2015). The plant is known as "*keharouâ*" in Arabic, "*ricin*" in French, and locally in Nigeria as *Laraa* in Yoruba; *Ogilisi* in Igbo; *Zurman* in Hausa and *Kpamfani gulu* in Nupe (Ghnimi *et al.*, 2014; Muhammad *et al.*, 2015). It is a soft-wooden tree that thrives in the tropics and the Arctic. It is well known as the Egypt old oil plant and probably originates from either Africa (Ethiopia) or India (Heike *et al.*, 2019). The leaves are applied topically to treat headaches and fever, and a poultice or fomentation is used on ulcers, boils, and

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swellings (Padma and Rupal, 2014). The plant's leaf, root, and seed oil have all been utilized in Indian traditional medicine to treat liver-related illnesses (Azardmard *et al.*, 2011). The seed oil is frequently administered to their abdomens to relieve children's flatulence. A decoction or poultice of leaves is used to stimulate the mammary glands.

In most developing countries, traditional medicines are a dominant part of their cultures. The remedies have considerable effectiveness, social acceptability, and economic viability and are mostly the only available source (Million *et al.*, 2019). In addition, the traditional system of herbal medicine has become a topic of global importance since they are considered rich sources of safe compounds useful in curing human diseases with rising cases of antimicrobial resistance (Abdulrasheed *et al.*, 2015). This situation has led to re-evaluating the therapeutic use of ancient remedies, such as plants and their extracts. With the increasing interest in and use of plant extracts, areas of concern that pose major challenges include their quality, safety, and scientific evidence concerning health claims. One possible strategy to curb the aforementioned problems is the systematic screening of natural products with the hope that it will result in the authentication of potency against tested strains and the development of new drugs to ensure an effective healthcare system in many developing nations, including Nigeria. This work was carried out to evaluate the antimicrobial activity of *Ricinus communis* seed oil and to add to the body of existing knowledge.

MATERIALS AND METHODS

Collection and authentication of plant materials

R. communis dried seeds were bought from the National Horticultural Research Institute in Ibadan. Mr. Lateef Akeem of the National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja, verified the seeds and assigned them the voucher NIPRD/H/7196. The same department received the voucher specimens for deposit.

Processing of plant materials

The dried *R. communis* seeds were cracked open, and the kernels were air-dried for three weeks. Using an electric blender, the dried kernels were grounded, resulting in a sticky paste that was then placed in clean polythene bags in preparation for soxhlet extraction.

Soxhlet extraction

This was done using the technique described by Warra and Abubakar (2015). The ground kernel sample was placed into a porous thimble and loaded in a Soxhlet extractor. N-hexane, which has a boiling point between 40-60 °Celsius, was used as the extraction solvent for 6 hours each time until the desired amount was produced. Using a water bath set at 55°C, the surplus solvent from the extracted oil was evaporated.

Quantitative phytochemical analysis

R. communis seed oil was subjected to quantitative phytochemical analysis as described by Oloyede (2005) and Sailaja *et al.*, 2016:

Quantitative estimation of alkaloids

0.5g of the seed oil was weighed and dissolved in 5 ml of 96% ethanol:20% H₂SO₄ (1:1) and then filtered. 1 ml of the filtrate was added to a test tube containing 5 ml of 60% H₂SO₄ and allowed to stand for 5 minutes. After that, 5 ml of 0.5% formaldehyde was added and allowed to stand at room temperature for 3 hours. The absorbance was read at a wavelength of 565nm.

Quantitative estimation of flavonoids

1 ml of the seed oil and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min, 0.3 ml of 5 % Sodium nitrite and 0.3 ml of 10% Aluminium chloride were added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically.

Quantitative estimation of saponins

Seed oil was dissolved in 80% methanol, 2 ml of Vanilin in ethanol was added and mixed well, and the 2 ml of 72% sulphuric acid solution was added, mixed well, and heated on a water bath at 60°C for 10 mins, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

Quantitative estimation of phenols

Different concentrations of the seed oil were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 mins, 4 ml of sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 mins at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer.

Quantitative estimation of tannins

In a 50 ml plastic bottle, 500 mg of the seed oil was weighed and poured, to which 50 ml of distilled water was then added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filtered was pipetted into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The seed oils were subjected to GC/MS analysis on a GC/MS system comprising an AOC- 20i autosampler,

and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions Restek RtxR – 5 (30 meter X 0.25 mm)(5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min, and an injection volume of 1.0 µl was employed (split ratio of 10:1); injector temperature 280 °C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 6°C / min to 280°C, then ending with an isothermal for 15 minutes at 280°C. Mass spectra were taken at 70 eV, 0.5 seconds of scan interval, and fragments from 40 to 550 Da. Total GC running time was 60 minutes (Shahid *et al.*, 2016).

Identification of compounds

National Institute of Standards and Technology (NIST) database was used to interpret mass spectrum GC/MS. The unknown components' mass spectra were compared with those of the known components stored in the NIST library.

Antimicrobial assay

Test micro-organisms

Two fungal isolates and three bacterial isolates were obtained from the Department of Microbiology, Federal University of Technology, Minna, Niger state. The bacterial isolates were *Escherichia coli*, *Salmonella* sp, and *Staphylococcus aureus*, and the two fungal isolates were *Aspergillus flavus* and *Aspergillus niger*.

Confirmation of bacterial isolates

The morphological characteristics of the bacteria were observed after 24 hours of growth on nutrient agar (NA) and confirmed by Gram staining and biochemical tests described by James and Chad (2019).

Confirmation of fungal isolates

The fungal isolates were confirmed based on their macroscopic characteristics after 72 hours of growth on potato dextrose agar (PDA) and their microscopic characteristics, as Hunter and Bamett (2000) described.

Inoculum preparation

Bacterial isolates were inoculated into nutrient broth and incubated at 37°C for 24 hours, and fungal isolates were inoculated in potato dextrose broth at 25°C for 72 hours. All cultures were standardized before usage.

Preparation of extract concentration

To get a 200 mg/ml concentration, 1000 mg of seed oil was weighed and dissolved in 5 ml dimethyl sulfoxide (DMSO).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the seed oil was determined using the method of Idris *et al.* (2019). The

prepared oil extract was mixed with two ml of sterile nutrient broth in a test tube. The extract concentrations (in mg/ml) of 100, 50, 25, and 12.5 were achieved by doubling dilution. Following that, 0.1 ml of the 0.5 McFarland standard of the isolates culture was added to each test tube, and the bacteria were then incubated for 24 hours at 37°C and fungal isolates were incubated for 72 hours at 25°C in potato dextrose broth. 2ml of 5% DMSO was used as negative control, and ciprofloxacin and fluconazole were used as positive controls for bacteria and fungi, respectively. After incubation, the tubes were examined for microbial growth by observing the turbidity, and the MIC was recorded as the lowest concentration, demonstrating no apparent growth.

Determination of Minimum Lethal Concentration (MLC)

The tubes that showed no signs of turbidity were subcultured into new NA and PDA plates and incubated at 37 °C for 24 hours (bacteria) and 25 °C for 72 hours (fungi), respectively. The MLC was determined by recording the plates that showed no growth.

RESULTS

Amber coloured oil was obtained from *R. communis* seeds, liquid at room temperature.

Quantitative phytochemical analysis of *R. communis* seed oil

Table 1 and Figure 1 shows the result of quantitative phytochemical analysis of *R. communis* seed oil. The result revealed the concentration of alkaloid, flavonoid, phenol, saponin, and tannin present in the seed oil.

Fatty acid components of *Ricinus communis* seed oil

The Gas Chromatography-Mass Spectrometer analysis showed the presence of 11 different fatty acids in *R. communis* seed oil, as shown on the total ion chromatograms in Figure 2 and Table 2, accounting for 94.29% of the oil. The most abundant fatty acid in *R. communis* seed oil was ricinoleic, constituting about 74.05%. Other fatty acids are palmitic acid 4.8%, oleic acid 5.43%, stearic acid 3.6% and linoleic acid 6.41%.

Colony morphology, Gram reaction, and biochemical characteristics of bacterial isolates

Colony morphology, Gram reaction, and biochemical characteristics of bacterial isolates are presented in Table 3.

MIC and MBC/MFC of *Ricinus communis* seed oil

Table 4 displays seed oil's MIC and MLC values against the isolates, which range from 12.5 to 100 mg/ml.

Morphological characteristic of fungal isolates

Macroscopic and microscopic characteristics of fungal isolates are presented in Table 5.

Table 1: Quantitative phytochemical analysis of *Ricinus communis* seed oil

Phytochemical	<i>R. communis</i> seed oil (mg/100g)
Saponins	99.48
Flavonoids	56.06
Tannins	343.7
Phenols	36.02
Alkaloid	308.44

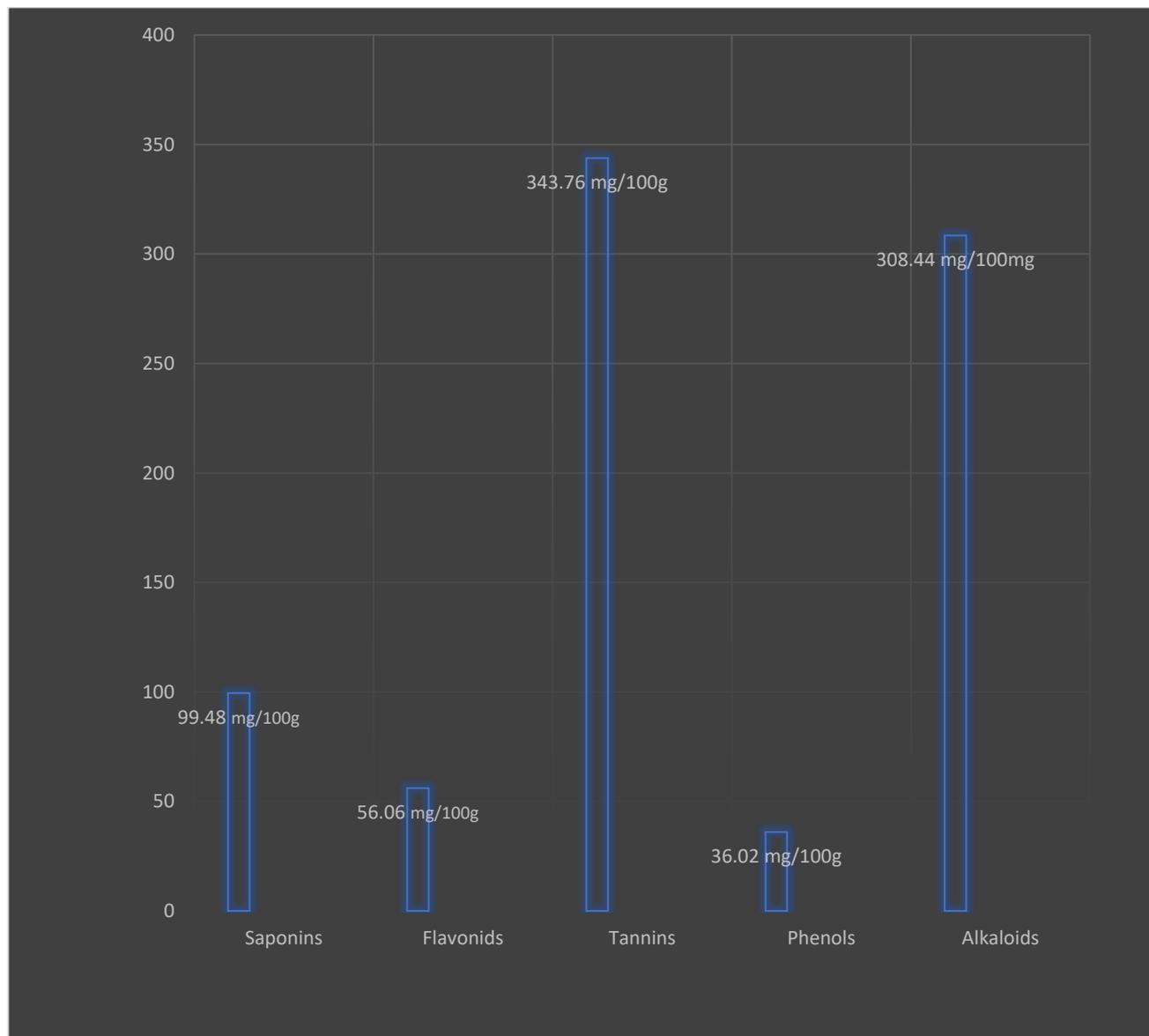


Figure 1: Bar chart showing values of phytochemical components

Table 2: Fatty acid components of *Ricinus communis* seed oil

Peak	Fatty Acid	Trivial Name	Retention Time	%Area
1	n-hexadecanoic acid	Palmitic Acid	14.930	4.82
3	9,12-Octadecadienoic acid	Linoleic Acid	16.330	6.41
4	9-Octadecenoic acid	Oleic Acid	16.387	7.43
5	Octadecanoic acid	Stearic Acid	16.588	3.60
6	12-hydroxy-9-cis octadecenoic acid	Ricinoleic Acid	17.830	74.05

Table 3: Colony morphology, Gram reaction, and biochemical characteristics of bacteria isolates

CM	GMR	CA	CO	CU	IN	OX	UR	Bacterial isolates
Whitish with glistening edges	+ Cocci	+	+	+	-	-	+	<i>Staphylococcus aureus</i>
Greyish white with moist surface	- Rod	+	-	-	+	-	-	<i>Escherichia coli</i>
Greyish white with smooth surface	- Rod	+	-	-	-	-	-	<i>Salmonella</i> sp.

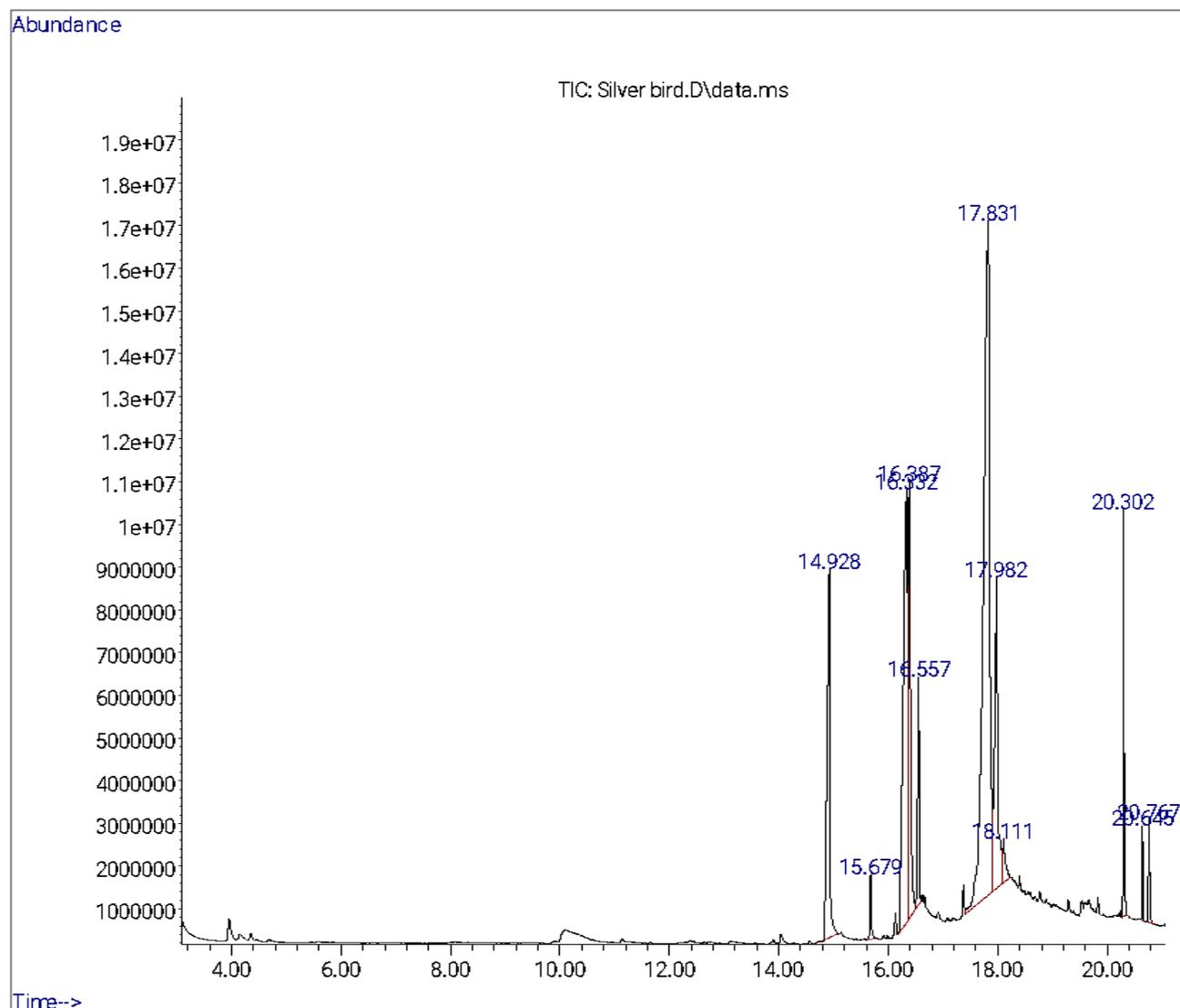


Figure 2: Total Ion Chromatogram of *Ricinus communis* Seed Oil

Table 4: MIC and MLC of *R. communis* seed oil

Isolates	MIC	MLC
<i>Escherichia coli</i>	25 mg/ml	50 mg/ml
<i>Salmonella typhi</i>	25 mg/ml	50 mg/ml
<i>Staphylococcus aureus</i>	12.5 mg/ml	50 mg/ml
<i>Aspergillus niger</i>	50 mg/ml	100 mg/ml
<i>Aspergillus flavus</i>	50 mg/ml	100 mg/ml

Table 5: Morphological characteristics of fungal isolates

Fungi	Macroscopic Features	Microscopic characteristics
<i>Aspergillus niger</i>	Black cottony colony with pale yellow edges producing radial fissures	Dark brown conidia with long conidiophores and globose vesicles
<i>Aspergillus flavus</i>	Yellowish Green woolly colony with cream colour edges	Globose conidia with rough conidiophores with phialades radiating from all sides

DISCUSSION

The Soxhlet extraction of *R. communis* seed produced an amber-colored oil that was liquid at room temperature. Joshua *et al.* (2020), Silma and Daneshwar (2016), and Pushapalata *et al.* (2014) have reported the presence of alkaloid, tannin, flavonoid, terpenoid, and phenol in *R. communis* seed oil. Except for flavonoids, Hashem *et al.* (2015) found that the *R. communis* seed extract included alkaloids, tannin, saponin, and phenol. Saponins are surface-active foaming agents that can function as fungicides and hydrolyzers (Helena and Leila 2016). Because of their ability to bond with cations and anions, saponins interact easily with cell membranes and, thus, disrupt their functions. Flavonoids confer antimicrobial activities by inhibiting nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, and inhibition of the porin on the cell membrane (Yixi *et al.*, 2015). Tannins are found in many different types of plants and have a wide range of biological properties, such as immune modulation, antibacterial, antiviral, anti-parasitic, and antioxidant properties (Qianqian *et al.*, 2017). Tannins have been found to inhibit microbial growth using different mechanisms of action, including iron chelation, cell wall synthesis inhibition, cell membrane disruption, and fatty acid biosynthetic pathways (Arakkaveetil *et al.*, 2020). The mechanisms of action of phenolic compounds on bacterial cells have been partially attributed to damage to the bacterial membrane, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation (Maria *et al.*, 2018). About 20% of the known secondary metabolites found in plants are alkaloids. They protect plants from predators and regulate their growth. Upon ingestion, they can disrupt protein function (Hélio and Arthur, 2015; Michael *et al.*, 2021). The discrepancy in the phytochemical component between this study and other studies may be because plants produce most phytochemicals to counteract threats; therefore, the type and intensity of threats will determine how different the phytochemicals are produced.

In this study, the component of *R. communis* seed oil agrees with the findings of Warra (2015) and Yusuf *et al.* (2015). Omowanle *et al.* (2018), in their work Physico-chemical and GC/MS analysis of some selected plant seed oils, castor, neem, and rubber seed oils, reported 74.45% of ricinoleic acid but in contrast with the findings of Manal *et al.* (2018) and Mohammed and Awatif (2018), higher value of ricinoleic acid 87% and 85% were recorded respectively. The presence of oleic acid in vegetable oils

has numerous health benefits. It is used in the treatment of several human diseases, such as diabetes, skin cancer, renal disease, heart attack, lupus, high blood pressure, and high cholesterol levels (Huang *et al.*, 2020). Linoleic acid has been reported to have anti-inflammatory and moisture retention effects, so it is becoming more popular in the beauty industry (Omowanle *et al.*, 2018). Palmitic acid produces soaps, cosmetics, and release agents (Warra, 2015). Stearic acid is useful in producing detergents, soaps, and cosmetics such as shampoos and shaving cream products (Omowanle *et al.*, 2018). The differences in the values of fatty acids in this study and other studies may be because plant varies greatly in the amount and component relative to habitat, seasons, and their environment.

The antibacterial properties of *R. communis* seed oil were previously documented by Mohammed and Awatif (2018) against the three bacterial isolates used in this investigation. The activity of *R. communis* seed oil against *S. aureus*, *S. Typhi*, and *E. coli* was also reported by Silma and Daneshwar (2016), and the outcome concurs with the result in the study of Shahid *et al.* (2016). The investigation of Momoh *et al.* (2012) reported the oil's antifungal activity against the fungal isolates. The result also agrees with the findings of Hashem *et al.* (2015) and corroborates its use in traditional medicine against ailments associated with the tested isolates. The antimicrobial activity of the seed oils may be due to the presence of bioactive compounds in the seed oil previously documented.

CONCLUSION

In this study, it can be concluded that the seed oil contained phytochemicals that exhibited antimicrobial activity and validated the use of seed oil in ethnomedicinal practices. Further studies should be carried out on other microbial strains and the safety of use of usage.

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