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Antimicrobial And Phytochemical Properties Of Castor Seed Oil On Arcobacter butzleri and Escherichia coli

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Abstract
Background*: Castor plant (Ricinus communis) is a species of flowering plants in the spurge family, Euphorbiaceae. The oil from castor seed has been used in clinical settings against numerous medical conditions such as liver diseases, diarrhea, constipation, intestinal obstruction, and skin diseases that are mainly caused by pathogenic organisms. The aim of this study was to identify the active components and antimicrobial effect of castor seed oil extracts on Arcobacter butzleri and stock cultures of E. coli isolated from poultry meat (chicken and turkey) in Osogbo, Nigeria.*
Methodology: *Cold extraction method was used to extract oil from castor seed beans using n hexane as solvent. The active components of the oil were determined using High Performance Liquid Chromatographic (HPLC) analysis. Ten (10) isolates of Arcobacter butzleri were identified and confirmed by the multiplex Polymerase Chain Reaction (mPCR), and two previously identified clinical isolates from stock culture of E. coli. Antimicrobial susceptibility testing of both the laboratory extracted castor seed oil and commercial castor seed oil on the test organisms were carried out using the agar well diffusion method.*
Results: *The HPLC analysis on the laboratory extracted castor seed oil revealed the presence of different fractions of Fatty acids and Phospholipids. The growth of all the Arcobacter butzleri and E. coli isolates were most inhibited at castor oil dilution of 50 mg/ml, followed by 25 mg/ml and then 6.25 mg/ml by zone diameter with no inhibition at 100 mg/ml on both extracted and commercial castor seed oil. It was observed that commercial castor oil showed lower inhibitory effect on all the isolates with an average of 7.1 mm and 8.0 mm compared to the laboratory extracted castor oil having an average of 14 .8 mm and 11.5 mm respectively.*
Conclusion: *This study showed that castor seed oil has inhibitory effect on Arcobacter butzleri and E. coli which is an indication that the oil contains antimicrobial components.*
Key words*: Phytochemical properties. Antimicrobial components. Polymerase Chain Reaction.*

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I. Introduction

Plants have been a major source of medicine and secondary metabolites have been ascribed for most plants' therapeutic activities (Mushtaq *et al*., 2018). It has been recorded that there are over 65,000 species of flowering plants that contain medicinal properties (Adhikari & Paul 2018, Akerele *et al*., 2007; Joshi & Sahu, 2014). From inception, large numbers of natural plants and their products are being used in prevention, treatment, elimination of psychological or social changes and investigating procedure (Shaikh *et al.*, 2016). The whole plant and its organs like stem, root, stem bark, leaves, flower, and excretory plant products (gum, resin, and latex) and seed are being used in production of antimicrobial agents. In African, medicinal plants constitute a rich but still largely untapped pool of natural products. Many countries from the developing world are still depending on medicinal plants for treatments. Nigeria is one of the countries that are richly blessed with natural plants with various medicinal values and antimicrobial potentials that are greatly supported by its climatic condition (Shakya 2016), castor plant is one of the valuable medicinal plants in Nigeria.

The Castor plant also known as *Ricinus communis* or Palma Christi, belongs to Family Euphorbiaceae, is an ancient annual woody plant that has several stems with green or reddish leaves color (Sarfaraz Khan Marwat *et al*., 2017). It is called tamil amanakku in Indian, carrapateriro/ mamona in Brazil, gulo in Ethiopia and in Nigeria it is known as ilara / iru / lapa pupa in Yoruba and Hausa it is called cika-gidaa. Its seed is known as castor bean which is not a true bean and is the only known source of the toxin ricin (Shankar & Joshi, 2021).

It is also the source of castor oil which is harvested to produce many beneficial products such as lubricants, pharmaceuticals, cosmetics, paints, and plastics (Shekade *et al*., 2022). Although an extremely potent protein toxin that is lethal to humans (Coopman *et al*., 2009) and animals (Aslani *et al*., 2007; Mouser *et al*.,

2007), ricin has also been investigated for use in the treatment of cancer and AIDS (Levitsky & Dembitsky, 2014), the toxin is deactivated if the extraction is carried out under heated conditions such as those found in castor oil production (Audi *et al*.,2005). The oil from castor seed is colorless or faintly yellow and almost odorless viscid liquid, having a nauseating taste. It is static and dries slowly, having a specific density of 0.959. (Patel *et al*., 2016).

The oil has been used in clinical settings against numerous medical conditions such as liver disease, diarrhea, constipation, intestinal obstruction, skin disease and many others, (Odungbemi, 2006; Blot *et al*., 2012; Joshi & Sahu, 2014).*Arcobacter* species are differentiated from campylobacters by their ability to grow in aerobic conditions at lower temperatures such as 15–30^{o}C (Ajebesone &Aina, 2004; Score & Phillips, 2015).*A butzleri* have been isolated from various retail foods (Irati Martinez-Malaxetxebarria *et al*., 2022) and animals (including pigs, cattle, and sheep) in association with abortion, reproductive problems, mastitis, gastric ulcers, and enteritis (Chieffi *et al*., 2020b). It has been reported that chicken meat carries the highest prevalence of *Arcobacter,* and the most common species is *A. butzleri* followed by pork and other types of meats from animal origin (Šilha, *et al.*, 2015,Šilha *et al*., 2018, Vidal-Veuthey *et al*., 2021).

The pathogenic *E. coli* is divided into those strains causing disease inside the intestinal tract and others capable of infection at extra intestinal sites (Babak Pakbin *et al*., 2022). *E. coli* is easily cultured in the clinical laboratory, but the identification of the different pathogenic genotypes requires virulence gene detection methods. Susceptibility of castor oil has been tested against *E. coli* isolated from recurrent urinary tract infections (Al-Kuraishi *et al.*, 2013). The record also showed that castor oil has well moderate activity and antimicrobial potentials against disease causing bacteria such as *Shigella dysenteriae*, *Salmonella typhi*, *Staphylococcus aureus* and fungal pathogens viz: *Macrophomin aphascolma, Alternaria alternata, Curvularia lunata* (Momoh *et al*.,2012; Dulal *et al*., 2021).

Globally, unprecedented increase of drug resistance by pathogenic micro-organisms as well as the appearance of undesirable side effect of certain antibiotics has been witnessed in the last two decades (Medina & Pieper, 2016) and other limitations of modern chemotherapeutic drugs, their high cost and non-availability, especially in underdeveloped area. As a result of this, it is necessary to have alternative means for new organic molecules with antimicrobial activity, which in addition could be potential sources for initial materials for the semi-synthesis of new developed drugs.

Arcobacter and E. coli species are Gram-negative, non-spore forming bacteria. They can be found in the intestinal tract of humans and animals, always been contracted through contaminated food or water and present with symptoms of acute gastroenteritis such as abdominal pain, acute or even prolonged diarrhea for up to several weeks (Van den Abeele, 2014).

II. Material and Methods

Seed collection and oil extraction from castor seed: Castor seed was plucked into a clean polythene bag from Ofatedo area, via Osogbo, Osun state, Nigeria. It was taken to the department of Botany in Obafemi Awolowo University, Ile Ife, Osun State, for proper identification. The castor seed was air dried at room temperature for about one week, the shell was separated from the bean and then crushed into paste (cake) with the aid of mortar and pestle to release the castor fat for the extraction process ((Ogunniyi, 2006; Yusuf & U. Agunwa, 2015).
Oil was extracted from 500 g of castor seed paste with one liter (1L) of n- hexane as a solvent, the paste was packed inside the muslin cloth and placed inside the covered conical flask, the mixture was homogenized using orbit shake apparatus (stuart) for four days at 200 rpm. The oil was separated from the solvent (Alabi, 2015) the total oil recovered from 500 g of castor seed paste after extraction was 10.1 ml.

Isolation of *Arcobacter* species from Poultry Meat

The twenty-five (25 g) of poultry meat sample was weighed and homogenized together with 125 ml of sterile Arcobacter broth with Cefoperazone, Amphotericin B, Teicoplanin selective (CAT) as the supplement (Oxoid, United Kingdom). After the thorough homogenization, red sheep blood was added as enrichment, and incubated for 48 hours at 30°C. Further passive filtrate of one hundred micro liter (100 µl) of enriched suspension, through a membrane filter with pore diameter of 0.45 µm, was done (Pall, USA). Passive filtration of the bacterial micro flora was incubated for 30 minutes at 25°C, followed by removing of the filter from agar media and then incubated at 30°C for 48 hours. Suspected colonies of *Arcobacter* Spp were streaked on arcobacter selective agar and incubated for another 48 hours, after which it was identified by the multiplex polymerase chain reaction (PCR) method (Houf, *et al*., 2000; Hrušková, *et al*., 2013).

PCR conditions.

The boiling method was used to extract DNA (Houf *et al.*, 2000). PCR reactions were performed in a reaction mixture of 20 µl as the final volume (Houf *et al.*, 2000). PCR involved 32 cycles of initial denaturation phase (95^{o}C-15 min), Denaturation phase (94^{o}C - 45 min) primer annealing (61^{o}C - 45 min) and chain extension (72^{o}C - 60 sec), final elongation phase (72^{O}C - 10 min).

Castor Seed Oil Preparation

Double fold dilution of the oil was made using 6 ml of crude extract as 100%, 3 ml of N- hexane were dispensed into different 4 sterile test tubes, 3 ml from crude extract was dispensed into the first tube as 50% and was mixed properly, from this tube another 3 ml was measured and dispensed into another tube of n- hexane making 25%, 12.5%, and 6.25% respectively.

Antibiotics Susceptibility Testing

A sterile swab was dipped into the inoculums' tube of the test organism and pressed against the inner part of the tube to remove excess fluid. The entire surface of the Muller Hinton (MH, Oxoid) agar plate was streaked evenly in three dimensions rotating the plate to ensure even distribution and was allowed to incubate for 30 minutes. A sterile forceps was then used to place antimicrobial discs on the plate and was pressed lightly to ensure contact with the agar. Antibiotic disc used were Ciprofloxacin (CIP) (10 µg), Oflaxacin (OFL) (5 µg), Tetracycline (30 µg), Gentamicin (GN) (10 µg). Ticarcillin (TIC) (75 µg) and Clindamycin (DA) (2 µg). The discs were placed at a distance from the edge of the plate and from one disc to another. The plates were then incubated at 30℃ for 48 hours and 30℃ and 37℃ respectively for 48hours and 24 hours aerobically.

Castor Seed Oil Susceptibility Testing on *Arcobacter butzleri* and *E. coli*

Agar well diffusion method was used. Muller Hilton agar was prepared according to manufacturer specification, sterilized, poured into petri dishes, and allowed to solidify. A sterile core borer of 6 mm diameter was used to make a well on the previously sealed agar with the studied organisms. 50 µl of different concentration (100, 50, 25, 12.5 and 6.25) (Dulal *et al*., 2021) of oil was dispensed into each well and incubated for 48 and 24 hours at 30^{o}C and 37℃ respectively. (Al-Kuraishi *et al*., 2013). Zones of inhibition were measured with transparent measuring ruler.

Results and Discussion: Inhibitory effect of both extracted and commercial castor seed oil as shown in table 1 and 2, The two bacterial isolates namely, *Arcobacter butzleri and E. coli* for the study showed significant inhibition to both extracted and commercial castor seed oil. The concentration of 50 µg/ ml of the oil has the highest zone of inhibition, followed by 25 µg/ml and the lowest was 6.25 µg/ml. There was no inhibitory effect to the two isolates at 100 µg/ml.

Table 1: Inhibitory effect of Extracted Castor seed oil on *A butzleri* and *E coli* using agar well diffusion method.

Organisms	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	P-value
C1	14.33±1.53	10.33±1.15	3.33±0.58	2.67±0.58	0.0725
C2	12.00±2.65	7.00±1.00	3.33±0.58	2.67±1.15	0.0615
C3	14.00±3.61	12.67±3.51	6.67±2.08	3.00±0.00	0.0389
C4	14.67±2.31	11.67±2.08	10.00±2.65	3.67±1.53	0.023
C5	13.00±2.65	6.67±2.08	3.33±0.58	3.67±0.58	0.0588
S	12.00±2.65	12.67±2.52	3.33±0.58	2.67±1.15	0.0474
T1	13.00±2.00	14.33±3.06	4.00±1.73	3.33±0.58	0.0278
T2	15.33±3.06	13.00±2.00	5.67±1.53	3.33±1.53	0.0168
T3	14.67±1.53	12.33±3.21	6.00±2.00	5.00±2.00	0.0312
T4	13.00±3.00	11.00±1.73	7.33±1.53	4.67±1.15	0.0147
T5	12.33±2.52	10.00±1.73	7.00±1.73	2.67±0.58	0.0361
E161	12.33±1.53	11.00±2.65	7.00±2.00	2.33±0.58	0.0657
E166	8.67±2.08	6.67±2.08	5.67±0.58	3.00±1.00	0.0584

KEY: Turkey (T1-T5), Chicken (C1- C5), S-standard strain of *Arcobacter butzleri*, *E. coli*(E166 & E161)

Table 2: Inhibitory effect of commercial Castor seed oil on *A butzleri* and *E coli* using agar well diffusion method.

Organisms	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	P-Value
C1	5.67±1.15	3.67±1.15	3.00±0.00	2.33±0.58	0.0147
C2	8.67±1.53	6.33±1.53	4.33±0.58	2.67±0.58	0.0239
C3	4.33±2.52	3.67±1.15	3.00±1.00	2.33±0.58	0.0045
C4	6.00±2.00	4.33±1.15	3.33±0.58	4.33±1.53	0.0039
C5	6.67±2.31	4.67±0.58	3.67±1.15	3.00±1.00	0.0111
S	5.00±1.00	4.00±1.73	4.33±1.15	3.00±1.00	0.0023
T1	5.33±2.08	4.00±1.00	5.33±1.53	2.67±0.58	0.0065
T2	5.33±2.52	5.33±1.53	4.33±1.53	2.00±0.00	0.0124
T3	6.00±1.73	6.33±2.52	3.33±1.53	3.00±1.00	0.0127
T4	6.67±2.08	6.00±2.00	4.00±1.00	3.33±1.53	0.0081
T5	5.67±2.08	4.33±1.15	2.67±1.15	2.00±0.00	0.0214
E161	5.33±3.21	5.00±2.00	4.00±	0.00±	0.0171
E166	5.33±0.58	4.67±0.58	3.33±0.58	0.00±	0.007

KEY: Turkey (T1-T5), Chicken (C1- C5), S-standard strain of *Arcobacter butzleri*, *E.coli* (E166 & E161).

HPLC analysis on castor seed oil: Fatty acids was fractionalized and shows the presence of Ricinoleic acid being (predominant) followed by Linoleic acid and Oleic acid (moderate); and traces of others such as Lauric acid, Myristic acid, Palmitic acid, Palmitoleic acid, Margaric acid, Stearic acid,Linolenic acid, Arachidic acid, Arachidonic acid, Behenic acid, Erucic acid and Lignoceric acid as shown in table 3.
Phospholipids were fractionalized and show the presence of Asphosphatidylethanolamine, Phosphatidylcholine (predominant), followed by Phosphatidylserine and Lysophosphatidylcholine (moderate) and traces of Phosphatidic acid as shown in table 4.

Table 3: Phospholipids analysis of castor seed oil.

GROUP NAME	AMOUNT (mg/100g)	Retention time (Min)
Phosphatidylethanolamine	115.53	14.16
Phosphatidylcholine	265.62	15.39
Phosphatidylserine	82.97	16.54
Lysophosphatidylcholine	16.01	17.70
Phosphatidylinsitol	54.21	18.68
Phosphatidic acid	28.14	19.67

Table 4: The fatty acid analysis of castor seed oil.

GROUP NAME	AMOUNT **(**mg/100g	Retention time (min)
Lauric acid methyl ester	0.03	12.82
Palmitoleic acid methyl ester	0.01	16.67
Margaric acid methyl ester	0.10	17.36
Stearic acid methyl ester	1.32	181.05
Oleic acid methyl ester	7.97	18.93
Linoleic acid methyl ester	10.87	19.10
Ricinoleic acid methyl ester	76.97	19.51
Linolenic acid methyl ester	0.44	20.65
Arachidic acid methyl ester	0.19	21.90
Arachidonic acid methyl ester	0.06	22.99
Behenic acid methyl ester	0.04	23.96
Erucic acid methyl ester	0.07	24.88
Lignoceric acid methyl ester	0.20	25.61
Myristic acid methyl ester	0.35	14.43
Palmitic acid methyl ester	1.38	16.03

III. Results and Discussions:

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in cost is not a simple task, there is little doubt that emergent antibiotics resistance is a serious global problem. The oil from laboratory extracted castor seed and commercial castor seed oil was evaluated for *in vitro* antibacterial activity against *Arcobacter butzleri* and *E. coli* indicating different zones of inhibition, The results revealed that n hexane extract of castor seed oil showed significantly higher inhibitory activity against *A butzleri* and *E. coli* compared to commercial castor seed oil which shows lesser zone of inhibitory activity against *A butzleri* and *E. coli*

From this study, the growth of *Arcobacter butzleri* and identified clinical isolate of *E. coli* were inhibited most at oil dilution of 50 mg/ml, 25 mg/ml respectively with zone of inhibition at average of 14.8 mm and 11.5 mm as against previous research done on effect of castor seed oil on *E. coli* with the zone of inhibition of 9.06 mm by (Al-Kuraishi *et al*., 2013;Vanitha Selvarajan *et al*., 2023) which could be as a result of the solvent concentration or method of extraction while there was no zone of inhibition at 100 mg/ml on both extracted castor oil and commercial castor which supported by "Rideal Walker" theory, (Nwanko *et al.*,2014).this was against the report of (Al-Kuraishi, *et al*.,2013) who reported that, as the concentration of the oil increases the wider the zone of inhibitions. (Al-Kuraishi *et al*., 2013)**.**

It can be clearly stated that castor seed oil is effective against food borne pathogens (Patel *et al*., 2016b; Can & Gerhard Buchbauer, 2015). And it has the potential to inhibit the growth of *Arcobacter and E. coli*, the antibacterial activity of this oil against *Arcobacter* and *E. coli* is significant because previous literature has been able to show the effect of other essential oils on this organism which has become an emerging food borne pathogen in Nigeria (Adesiji*et al*., 2011; Momoh *et al*.,2012; Santos *et al*., 2017)

From this study, the results revealed that n hexane extract of laboratory extracted castor seed oil showed significantly higher inhibitory activity at average of 14.8 and 11.5mm against *A butzleri* and *E. coli* compared to commercial castor seed oil which shows lesser zone of inhibitory activity at average of 7.01 and 8.0 mm against *A butzleri* and *E. coli* which is still within the range of alcoholic extracted castor oil in Iraq with the zone of inhibition of 9.06 mm in diameter as reported by (Al-Kuraishi *et al*., 2013).The difference in the inhibitory effect of both commercial castor oil and laboratory extracted castor oil on the tested isolates may be

due to the fact that the commercial castor oil has undergone through redefinition which might have led to the loss or destruction of some active components of the oil. However, the method used in extraction of commercial castor oil might also be different from the one used during this research. Furthermore, it was also observed that *Arcobacter butzleri* are more susceptible to castor seed oil compared to *E. coli* based on the diameter of zones of inhibition.

Antimicrobial susceptibility of antibiotics on both isolates of *A butzleri* and clinically identified isolates of *E. coli* was determined in which they were non susceptible to all antibiotics due to missing standardized protocols and missing clinical breakpoints, as well as epidemiological cut-off values EUCAST breakpoints for both *Arcobacter butzleri* and *E. coli* (ECOFFs), EUCAST breakpoints for C. coli was used (ECOFFs), (Van den Abeele *et al*., 2016), Also Clinical Laboratory Standard Institute breakpoint was used for *E. coli* where there are no available animal species specific breakpoints. (CLSI, 2016). The breakdown of each antibiotic used is as follows: Ciprofloxacin (≤.0.5), Ofloxacin (≤.0.5), Tetracycline (≤.2.0) and Gentamycin (≤ 2.0) but both Clindamycin and Ticarcilin had no inhibitory activities against all test isolates. This could be because of the number of sample sizes as against the previous work. Though, contrary to the report that all *A butzleri* were susceptible to Gentamycin. (Rahimi, 2014; Van den Abeele *et al*., 2016).

The experimental evidence observed in this research through HPLC analysis revealed that castor seed oil contained active chemical components such as fatty acid and phospholipids which could be responsible for the antimicrobial activities of the oil. Mostly, oils from the plant source are unsaturated. An unsaturated fatty acid consist of at least one double bond within the fatty acid chain. A fatty acid chain is monounsaturated if it contains one double bond, and polyunsaturated if it contains more than one double bond. Examples of unsaturated fatty acids are Palmitoleic Acid, Oleic Acid, myristoleic acid, Linoleic Acid, and Arachidonic Acid.

HPLC analysis on the laboratory extracted castor seed oil revealed the presence of different fractions of fatty acids which has been previously reported by (Hassan & Hetta, 2019; Román-Figueroa *et al.,* 2020) that each fatty acid fractions contained antimicrobial efficiency.

In conclusion, the presence of difference fractions mainly fatty acids, phospholipids and sterols in different plant extracts may justify the therapeutic properties of these herbal agents that were used by traditional healers again various diseases. This study shows that castor seed oil has inhibitory effect on *Arcobacter butzleri* and *E. coli* which is an indication that the oil contains antimicrobial components. The potential antimicrobial property of the castor seed oil could be harnessed for use in pharmaceutical companies to produce drugs against the studied pathogens, while further investigations can still be envisaged on phospholipids and sterols present in castor seed oil. More research needs to be done in future on the test organisms by incorporating a broad spectrum of antibiotics apart from the one used during this research, also the sample size needs to be increased to give it the benefit of doubt.

IV. Conclusion:

This study shows that antimicrobial potency of castor seed oil extracted through cold extraction method inhibit the growth of *Arcobacter butzleri* and *E coli* in addition to its purgative, anti-inflammatory, and cancer treatments recorded by previous researchers. It therefore feasible that castor oil can be part of components used in production of drug against the test organisms.

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