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Evaluation of the Antimicrobial and Phytochemical Properties of Oil from Castor Seeds (*Ricinus communis* Linn)

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ABSTRACT
The antimicrobial activity of the essential oil of castor (Ricinus communis) seeds extracted using soxhlet extractor in 98% n-hexane was assessed using in-vitro assay. Twenty microorganisms made up of fourteen bacteria and six fungi were used in the bioassay. Comparatively, bacteria were found to be more susceptible than fungi. The minimum inhibitory concentration (MIC) of the extract was found to range between 6.25 mg/ml and 12.50 mg/ml for bacteria while that of fungi ranged from 12.50mg/ml to 25.00mg/ml. Comparison of the antimicrobial efficacy of the extract and commercial antibiotics showed that the latter were more potent against the test organisms with the exceptions of erythromycin, ampiclox and rifampin group for Gram positive organisms and, septrin and ceporex group for Gram negative organisms respectively. The quantitative phytochemical screening showed that tannin, phenol, alkaloid, phytate, oxalate, saponin, cyanogenic glycoside and flavonoid were present in a decreasing order. The spectrophotometric data of the extract using ultraviolet radiation, infrared and H-NMR as well as carbon 13 NMR showed the presence of various compounds such as cineole, 2- octanol, terpenene -4-ol, limonene, sabinene, pinene, terpinene, and methyl groups in the oil.
***Key words**: antimicrobial, phytochemicals, castor oil, microorganisms.*

INTRODUCTION

The oils of medicinal plants have been used for treatment of various ailments since men learnt the art of extraction [1]. Clove oil for instance has been used for dental pain as an anodyne (painkiller), as antihelmintic and as aromatherapy when warming of the digestive system is needed as far back as 1721 BC [2].

Castor plant, *Ricinus communis*, is a species of flowering plant in the spurge family, Euphorbiaceae. Its seed is the castor bean which, despite its name, is not a true bean. Castor plant is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions [3]. Although monotypic, the castor oil plant can vary greatly in its growth, habitat and appearance. It is a fast-growing, suckering perennial shrub which can reach the size of a small tree (around 12 metres / 39 feet). If sown early, under glass, and kept at a temperature of around 20 °C (68 °F) until planted out, the castor oil plant can reach a height of 2–3 metres (6.6–9.8 ft) in a year. The flowers are borne in terminal panicle-like inflorescences of green or, in some varieties, shades of red. The oil from the castor seed is colourless or faintly yellow, almost odorless, viscid liquid, having a taste at first bland but subsequently avid and nauseating. It is fixed and dries very slowly, having a specific gravity, 0.958. It is slightly dextrorotatory, about + 4^{o} 30^{1}. It has a refractive index, 1.4790 to 1.4805 and solidifies at -10^{o} C to - 18^{o}C. Its acidity is expressed as oleic acid which is 1.5 percent. The oil extracted from the seed have been used in small doses in clinical setting for numerous medical conditions such as liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyper activity in children and to avert threatened abortion in pregnant women [4,5,6]. Traditionally, the Ebira people in Kogi State of Nigeria use it for skin diseases, purgative, heal irritated or inflammed nipples and to aid delivery in delayed expectant mothers. Although, much has been documented on the uses of castor oil, there is no report on its antimicrobial activity. This study therefore was designed to evaluate the antimicrobial and phytochemical properties of castor oil.

MATERIALS AND METHODS

Plant materials

The castor plant seeds used in this study were the white variety of the *Ricinus communis* L. This was collected from a farm at Eika village, Okene, Kogi state, Nigeria.

Microorganisms used in the bioassay

The microorganisms used were obtained from the Microbiology Department, University of Ibadan Teaching Hospital, Ibadan, Oyo State, Nigeria. The Gram positive bacteria used include: *Staphylococcus aureus, Bacillus cereus, Streptococcus faecium, Streptococcus pyogenes, Bacillus marcesene* and *Streptococcus mitis* while the Gram negative used were *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella enteritidis, Salmonella typhimurium, Klebsiella pneumoniae* and *Proteus vulgaris*. The fungi used are *Fusarium oxysporum, Penicillium oxalicum, Candida albicans, Penicillium cinirium, Aspergillus flavus* and *Aspergillus niger.*

Extraction of oil from the seeds

The soxhlet extraction of castor oil from 500g of castor seeds using one litre of n-hexane was done according to the method of Odugbemi, [6].

Antimicrobial sensitivity testing of the extracted oil.

With the aid of a sterile pipette, 1ml of 18 hour old peptone broth culture of the test organism cultured at 37˚C was added to 20ml sterile molten NA and PDA respectively which had already cooled to 45^{o}C. This was well-mixed and allowed to set. With the aid of sterile 4mm cork borer, 3 wells were bored on the agar surface. To each of the 2 wells was added 2 drops (0.4ml) of the oil using Pasteur pipette aseptically. The well in the center was filled with same amount of sterile distilled water to serve as control.

Antibiotic assay

The Optu-sensitivity discs were used for this assay. The discs were picked with sterile forceps and placed on the surface of the solidified NA previously seeded with 10^{6} an overnight bacterial culture. The plates were incubated at 37^{o}C for 24hours. The plates were then examined for clear zones of inhibition of bacterial growth around the discs. The procedure was repeated for test fungi. The antimycotic drug used was fulcin and incubation was done at 25^{o}C for 5 days. The results were then compared with that of oil extract.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) was determined using the method described by Olutiola *et al*. [7]. Standardization of inoculum size was determined using spectrophotometer and the plate count method. Different concentration of the extract were prepared at 25, 12.5, 6.25 and 3.1mg/ml, and 5ml of an 18hour old culture of the organism was pipetted into test tubes. Using sterile syringe, 1ml of the different concentrations of the extract was poured into the broth culture and incubated for 24hours at 37^{o} C. The tubes were checked for growth as indicated by turbidity and confirmed with the aid of spectrophotometer. The least concentration at which inhibition was noticed was taken as the minimum inhibitory concentration (MIC).

Phytochemical screening

Basic phytochemical analysis was carried out to determine the bioactive ingredient present in the extract and their percentages. The standard methods of analysis of analytical methods committee of Royal Society of chemistry, (2002) were adopted to determine cyanogenic glycosides, tannin, saponin, oxalate, phytate, phenol, alkaloid and flavonoid.

Spectrophometric analyses

Nuclear magnetic resonance, infra-red and ultra violet analyses of the oil extract was carried out in Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Osun State of Nigeria according to standard methods of analysis of analytical methods committee of Royal Society of chemistry, (2002).

Statistical analysis

The data gathered were processed using descriptive one way analysis of variance, SPSS Version 10 Microsoft Windows 7. The Duncan Multiple Range Test was used as a follow up test.

RESULTS AND DISCUSSION

***In vitro* inhibitory effect of castor oil on test organisms**

The extracted castor oil inhibited the growth of all the test organisms. Among the Gram positive bacteria, *Staphylococcus aureus* was the most sensitive and *Micrococcus luteus* was the least sensitive with zones of inhibition of 7.00 mm and 2.50 mm respectively. Among the Gram negative

bacteria, *Escherichia coli was the* most sensitive and *Proteus vulgaris* was the least sensitive with zones of inhibition of 6.50 mm and 3.00 mm respectively. Among the fungi, *Fusarium oxysporum* was the most sensitive while *Aspergillus niger* was least sensitive to the oil with zones of inhibition of 4.00 mm and 1.50 mm respectively. Generally, the oil was more effective on bacteria than fungi as shown in Table 1.

Table 1: Sensitive pattern of selected microorganisms to the extracted castor oil

Standard culture (cfu/mL)	Organism	Diameter of Zone of inhibition (mm)
2.6×10^6	*Bacillus cereus*	**4.00**
2.4×10^6	*Bacillus macerans*	**3.00**
3.6×10^6	*Micrococcus luteus*	**2.50**
3.0×10^6	*Staphylococcus aureus*	**7.00**
2.8×10^6	*Streptococcus faecium*	**4.50**
3.4×10^6	*Streptococcus mitis*	**5.00**
3.1×10^6	*Streptococcus pyogenes*	**5.50**
3.9×10^6	*Escherichia coli*	**6.50**
2.6×10^6	*Klebsiella pneumoniae*	**4.00**
2.9×10^6	*Proteus vulgaricus*	**3.00**
3.1×10^6	*Pseudomonas aeruginosa*	**4.50**
3.7×10^6	*Salmonella enteriditis*	**5.00**
3.5×10^6	*Salmonella typhimumium*	**4.50**
3.6×10^6	*Shigella dysenteriae*	**5.00**
3.0×10^5	*Aspergillus flavus*	**2.00**
4.0×10^5	*Aspergillus niger*	**1.50**
6.0×10^5	*Candida albicans*	**3.00**
2.0×10^5	*Fusarium oxysporum*	**4.00**
2.0×10^5	*Penicillium cinirium*	**2.50**
3.0×10^5	***Penicillium oxalicum***	**2.50**

Antibiotic sensitivity assay

The result of the antibiotic sensitivity assay on Gram positive bacteria is shown on figure 1. Some of the antibiotics were found to have higher antimicrobial activities on the organisms than the castor oil. Rifampin, lincomycin and floxapen had lower antimicrobial activities on the organisms than the extract while streptomycin, norfloxacin, chloramphenicol, gentamycin and ciproflox showed higher antimicrobial activities than that of the extract. Erythromycin and ampiclox had approximately the same effect as that of the extract on the test bacteria.

On the Gram negative bacteria, tarivid, streptomycin, nalidixic acid, gentamycin, augmentin and ciproflox showed higher antimicrobial activities than the extract. The result also showed that some of the test organisms were resistant to ampicillin, peflacine and ceporex making the extract more effective than the antibiotics as shown on figure 2.

Penicillum cinirum was most sensitive while *Candida albicans* was least sensitive to Fulcin with zones of inhibition of 12 mm and 4 mm respectively. However, in general, the antifungal agent (Fulcin) was more effective than the extract as shown in figure 3.

Minimum inhibitory concentration (MIC)

All the Gram positive bacteria had a constant MIC value (12.50 mg/ml) except *Staphylococcus aureus* that had a lower value of 6.25 mg/mL. The MIC for the fungal group were however higher. *Aspergillus flavus*, *Aspergillus niger* and *Penicillium cinirium* had 25.00 mg/mL as their MIC while *Candida albicans, Fusarium oxysporum* and *Penicillium oxalicum* had 12.50 mg/mL.

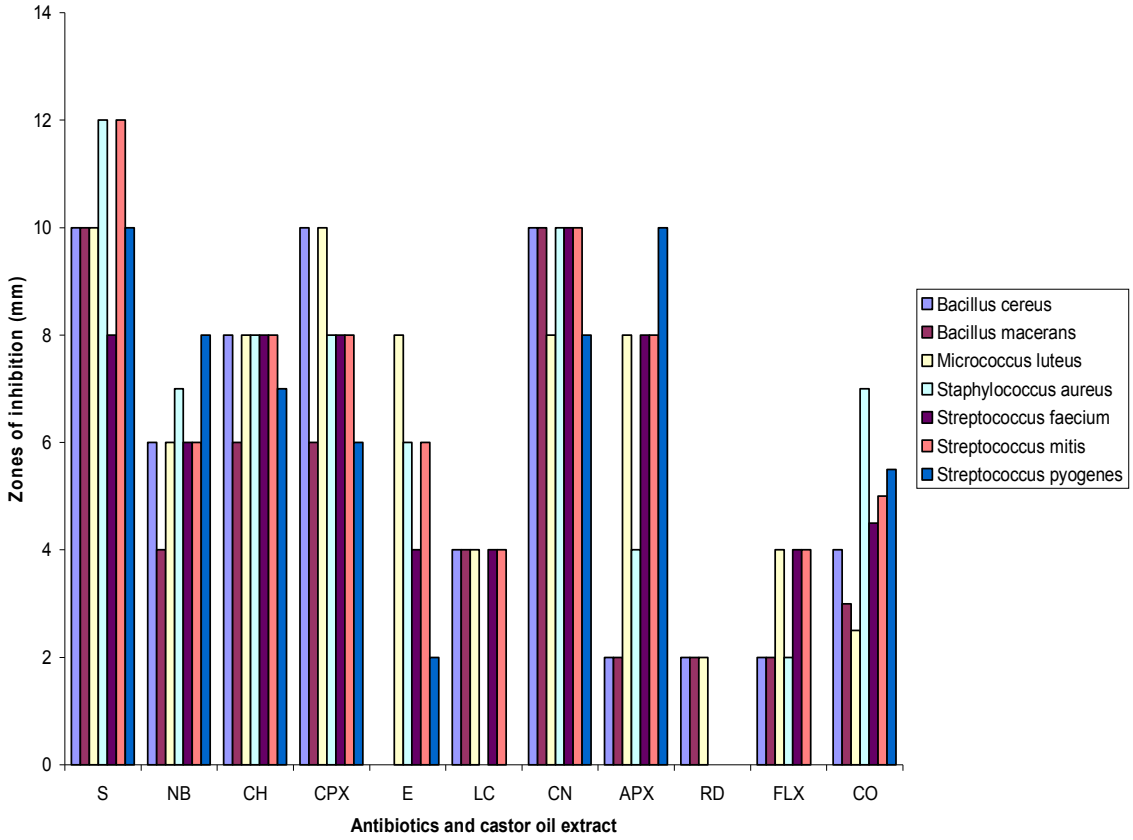

Figure 1: Bar –chart showing the comparison of the activities of extract and standard antibiotics on Gram positive bacteria.

Keys S = Streptomycin, NB = Norfloxacin, CH = Chloramphemicol, CPX = Ciproflox, E = Erythromycin, LC = Lincocin, CN = Gentamycin, APX = Ampiclox, RD = Rifampin, FLX = Floxapen

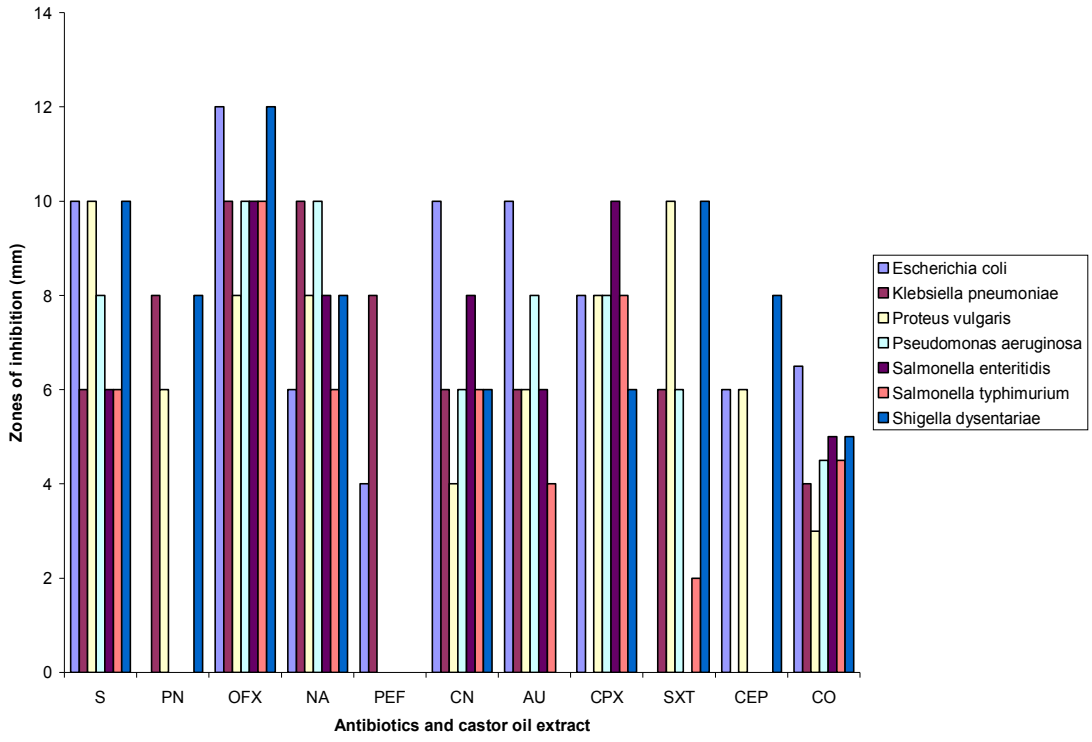

Figure 2: Comparative antimicrobial activities of castor oil extract and standard antibiotics on Gram negative bacteria. Keys S = Streptomycin, PN = Ampicillin, OFX = Tarivid, NA = Nalidixic acid, PEF = Peflacine, CN = Gentamycin, AU = Augmentin, CPX = Ciproflox, SXT = Septrin, CEP = Ceporex.

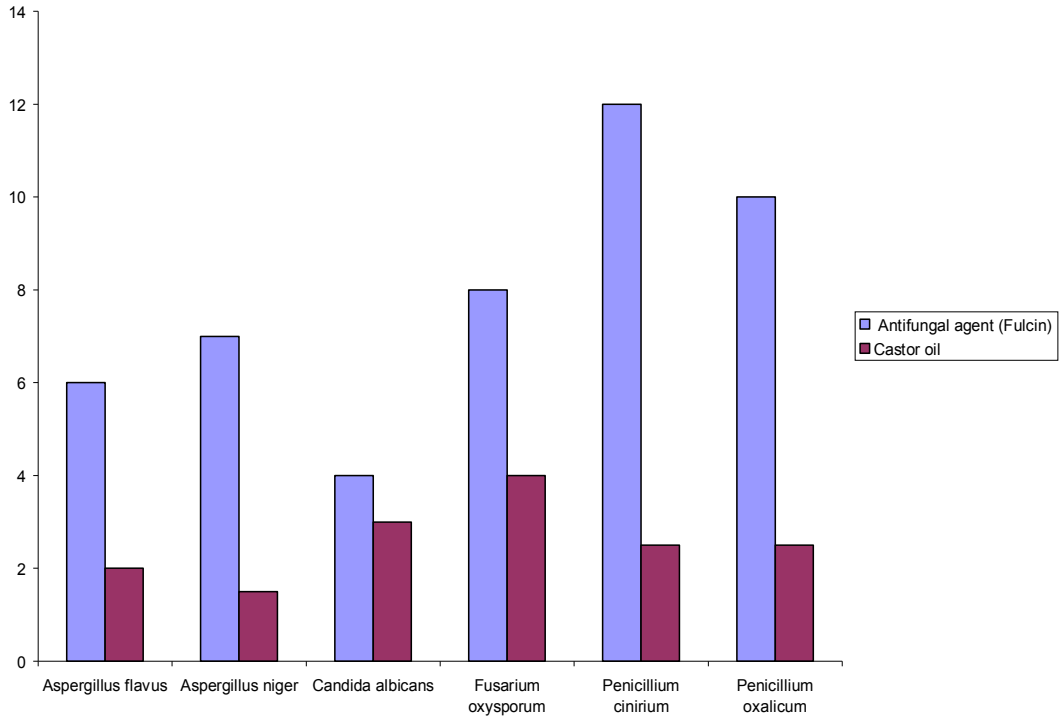

Figure 3: Comparative antimicrobial activities of extract and standard antifungal agent on selected fungi.

Phytochemical analysis

Phytochemical screening of the extract for the presence of bioactive compounds revealed the presence of tannin, saponin, alkaloid, phytate, oxalate, flavonoid, cyanogenic glycoside and phenol. The most abundant phytochemical present in the extract was tannin (0.35 %) while the least was observed in flavonoid and cyanogenic glycoside (0.03 %) each.

Ultra-violet Result

The following absorptions were observed: 220 nm, 226 nm and 236 nm with 226 nm being the lambda maximum. These absorptions may be as a result of conjugated double bond that is present in some of the fatty acid present. Minor absorptions were observed at 268 and 280 nm respectively.

Infrared Result

The Infrared result, at different wave number per centimeter and their probable functional groups are shown below in Table 2.

Nuclear magnetic Resonance Result

The result of the NMR shows different functional groups as well. The various frequencies and their corresponding identification is shown in Table 3. The proton NMR of the extract and its identification is shown in Table 4.

Table 2= Infra-red result

S/N	Wave number (cm^{-1})	Probable functional group
1	**3346**	**OH, C-H (stretch), COOH, NH**
2	**2693**	**O-H, CH**
3	**2879**	**O-H, C-H, CH_2, CH_3**
4	**1730.7**	**C=O, ketones**
5	**1646.8**	**C-H, CH_2, C-O**
6	**1437**	**CH2 (Stretch), C-H (bending)**
7	**1377.6**	**C-H, C-O, C=C**
8	**1078.5**	**C-H, CH2, C=C**
9	**1042.7**	**C-H (stretch), CH_2**
10	**875.1**	

Table 3: NMR frequencies and their identifications

Index	Frequency	Identification
1	8729.775	C = O
2	6630.101	CH
3	6316.905	CH
4	3590.839	CH-O
5	3470.673	CH-O
6	3124.287	CH_2
7	2882.047	CH_2
8	1838.695	CH_2
9	1762.017	CH_2
10	1714.714	CH_2
11	1706.321	CH_2
12	1598.743	CH_2
13	14.83.918	CH_2
14	1474.762	CH_2
15	1462.173	CH_2
16	1456.069	CH_2
17	1372.144	CH_2
18	1285.547	CH_2
19	1244.729	CH_2
20	1133.336	CH_2
21	889.188	CH_3
22	696.540	CH_3

Table 4: Proton NMR of the extract and their identifications

Proton NMR (ppm)	Identification
4.38 (broad)	OH Signals – Alcohols of Cineole and 2 – Octanol
3.29 – 3.45	CH = CH of Limonene, Sabinene, Pinene and Terpinene.
1.75 – 2.15	CH_2 (Methylene group of the essential oil)
0.80, 0.85, 0.90, 0.98, 1.00, 1.05	CH_3 Signals (Methyl group of the essential oil).

DISCUSSION

The findings in this research work indicate that the percentage yield of the extract using 98% N-hexane as solvent of extraction is about 20% of the total mass of the seed. This corroborate the report of Gerhard *et al,*. [8] that the amount of volatile oil in castor beans is 20%.

From the results of this investigation, the antimicrobial activities of the extract against test organisms highly varied. Bacteria were observed to be more sensitive than fungi. One reason for the low susceptibility of fungi is probably their eukaryotic nature, which is responsible for their advance cellular and molecular process, when compared to bacteria which are prokaryotic in nature.

The susceptibility of some of the organisms used may be due to their genetic make - up and absence of resistant transfer factor. *Streptococcus* species used showed moderate susceptibility to the extract and this may be due to their ability to produce different enzymes and toxins which may be able to degrade some of the active components of the essential oil. The *Bacillus* species showed low susceptibility to the extract probably due to their ability to form spores which could have shielded them from the extract. Fungi were less susceptible to the castor oil extract than bacteria, however none of the fungi tested for was resistant to the extract. Though, the mechanism of action of the extract was not studied, the presence of biologically active chemicals such as saponin, tannin, phenol, cyanogenic glycoside and flavonoids could be responsible for the antimicrobial activity of the oil. The presence of the various compounds revealed by the spectrophotic analysis of this

extracts shows that the antimicrobial properties of the essential oil could be traced to these compounds. According to Omidbeygi *et al,* [9] and Rota et al, [10], the composition, structure, as well as the functional group of an essential oil play an important role in determining its antimicrobial activity.

This study has been able to show that castor oil has antimicrobial activity in addition to its purgative, anti-inflammatory and labor inducing ability that has been documented earlier on by many researchers. It is therefore conceivable that castor oil could be used in treating infections caused by the test organisms used in this work in the absence of conventional therapy or antibiotics.

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