



Antimicrobial Activity of Cu, Ni Carboxylates of Castor (*Ricinus communis*) Seed Oil and Their Calcinated Derivatives

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ABSTRACT: Copper and nickel carboxylates of castor seed oil were prepared by metathesis in aqueous ethanol. The FTIR spectra of the carboxylates are characterized by two asymmetric vibrations of the carboxylate group and two symmetric vibrations. For Cu carboxylate, the values are 1594 & 1532 cm^{-1} and 1458 & 1409 cm^{-1} while those of Ni carboxylate were observed at 1561 & 1513 cm^{-1} and 1469 & 1412 cm^{-1} respectively indicating that the carboxylates existed as bridging bidentate structure and confirmed their formation. The carboxylates were calcinated in muffle furnace at 400°C to obtain blackish derivatives. Antimicrobial activities of the carboxylates and their calcinated derivatives were assayed using modified Kirby Bauer disc diffusion method against two groups of medically implicated bacterial species, viz: Gram positive organisms (*Staphylococcus aureus* and *Streptococcus faecalis*.) and Gram negative organisms (*Pseudomonas fluorescense*, *Salmonella thypii*, *Klebsiella pneumonia* and *Proteus mirabilis*). It was observed that the antimicrobial potencies of the carboxylates and their derivatives compared favourably with the standard antibiotics used for the assay. However, calcinated derivative of Ni carboxylate showed a quite significant increase in the antimicrobial potency against both gram negative and gram positive microorganisms when compared with its carboxylate. ©JASEM

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Metal carboxylates are salts of long-chain fatty acids with alkaline-earth or heavy-metals which are insoluble in water but soluble in non-aqueous solvents. Due to variation in the valency of the metal cations and alkyl chain length of the fatty acids, the physical properties of metal carboxylates vary considerably. Their solubility in organic solvents accounts for their use in producing a wide range of industrial products (Essien et al., 2012). Various methods have been reported for the preparation of metal carboxylates (Egbuchunam et al., 2007), these include precipitation and fusion. Precipitation method produces fine powder with a large surface area, however, fusion method produces flakes or pellets. Metal carboxylates have varied applications in industries and in our daily life. Those of barium, cadmium, lead, zinc and calcium have found application as thermal stabilizers

for poly(vinyl chloride) (Owen and Msayib, 1989; Bacaloglu and Fisch, 1994). Calcium and magnesium carboxylates are used as corrosion inhibitors in non-polar media; lead, manganese, cobalt and zinc carboxylates are used in paints to accelerate drying while copper carboxylate exhibits fungicidal properties (Salager, 2000). Silver carboxylates are used as the source of silver in thermographic and photothermographic materials (Binnemans et al., 2004). Some have found use in greases, cosmetics, fuel additives, drug formulation and textiles (Egbuchunam et al., 2007).

To date, microbial infection is one of the major problems worldwide, due to the increasing rate of resistance. Resistant pathogens have been found among both gram-positive and gram-negative bacteria and the high natural resistance to antimicrobials is frequently observed in

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many gram-positive bacteria (Suksrichavalit *et al.*, 2008). However, a number of bioactive metal complex based compounds such as anticancers, antioxidants and antimicrobials have been reported (Galal *et al.*, 2010; Liu *et al.*, 2010) and many are yet to be discovered.

Many seed oils that have been characterized are yet to be explored for the preparation of metal carboxylates despite being the most abundant source of fatty acids. A number of reports (Essien *et al.*, 2012; Folarin *et al.*, 2011; Egbuchunam *et al.*, 2007 and Okieimen *et al.*, 2006) on the characterization and properties of metal carboxylates prepared using seed oils are available in the literature, thanks to their abundance and low cost as starting materials for the preparation of metal carboxylates.

Literature survey revealed that castor plant seed is an important oil seed (40 – 60% oil) with ricinoleic acid as the major fatty acid of the oil (about 90%). The presence of OH group at C-12 of the fatty acid makes it a valuable chemical feedstock. This long-chain fatty acid and others present in the oil are suitable for preparing metal carboxylates. There has not been any report of copper and nickel carboxylates prepared from castor seed oil, hence, this work aims at preparing copper and nickel carboxylates of *Ricinus communis* seed oil, their calcinated derivatives and investigate associated antimicrobial activity.

MATERIALS AND METHODS

Preparation of the metal carboxylates: Castor oilseeds were collected at Abeokuta and the oil was extracted with hexane using Soxhlet extraction method. About 9.2 g of the oil was dissolved in 50ml of hot ethanol, followed by treatment with 20 ml of 20% (w/v) NaOH solution. Few drops of dilute HNO₃ was added to neutralize excess NaOH. To this mixture, 100ml of 30% (w/v) solution of the appropriate metal salt was slowly added with continuous stirring. The precipitated metal carboxylates were washed with warm water, air-dried and subsequently

dried in an oven at 60°C to constant weight (Folarin and Enikanoselu, 2010). Analar grade of CuSO₄.5H₂O and NiCl₂.2H₂O were used for the preparation.

Preparation of calcinated derivatives of the carboxylates: The calcinated derivatives of the carboxylates were prepared by the method described by Azam *et al.*, 2012. A known amount of carboxylate was put in a crucible and placed in muffle furnace at 200°C to char for 2 hr and subsequently heated at 400°C for 3 hr. Thereafter, the crucible with the blackish content was transferred into a desiccator to cool.

FTIR spectrum: The FTIR spectra of the carboxylates were measured using a Perkin-Elmer Spectrum 100 spectrometer equipped with IR microscopy accessory and a germanium crystal.

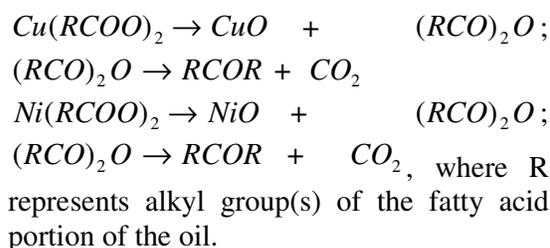
Antimicrobial susceptibility assay: Antimicrobial activities of Cu, Ni carboxylates and their calcinated derivatives were assayed against some bacteria of medical importance using modified Kirby Bauer disc diffusion method (Azam, *et al.*, 2012). Wells were cut into the agar using a 7 mm diameter sterile cork borer. The wells were sealed with one drop of molten agar (0.8% nutrient agar) to prevent leakage from the base of the seeded culture plates. The presence or absence of inhibition zone(s) around the wells was determined after incubation for 24 h. at 37°C. Standard antibiotic (Ampicillin) was used as a positive control, while two solvents blank were run as a negative control.

RESULTS AND DISCUSSION

The FTIR spectrum of the Cu carboxylates is shown in Figure 1 indicating the binding mode of the carboxylate groups to the metal ion. As shown in the figure, Cu carboxylate has two asymmetric carboxylate vibrations at 1594 and 1532 cm⁻¹ and two symmetric vibrations at 1458 and 1409 cm⁻¹ indicating that the copper carboxylate existed as bridging bidentate structure. This is

consistent with values reported for some carboxylates (Roy et al., 2006; Jona et al., 2001) and confirmed the formation of metal carboxylates. The CH₂ antisymmetric and symmetric stretching vibrations occur in the region 2918 and 2849 cm⁻¹ while the band at 3449 cm⁻¹ is ascribed to hydrogen bonded OH groups possibly from water or fatty acid portion of the carboxylate. Ni carboxylate displayed similar IR characteristic bands.

Some metal soaps of carboxylic acids have been reported to decompose at higher temperature by multiple steps leading to the formation of carboxylic acids, metal oxides, carbondioxide and ketone (Folarin et al., 2011). Azam et al reported the formation CuO nanoparticles by heating copper citrate in the temperature range of 400 – 700°C, the report however indicated that the nanoparticle produced at 400°C had the highest antimicrobial activity, this necessitated our choice of 400°C calcination temperature. The calcination process of the carboxylates is proposed as follows:



The blackish substances obtained after calcination are most likely to be CuO and NiO.

The antibacterial activity of Cu and Ni carboxylate and their derivatives elicited some levels of antimicrobial properties against some isolates of medical importance (Tables 1 and 2). Cu carboxylate inhibited both gram positive (*S. aureus* and *S. faecalis*) and gram negative (*P. fluorescence*, *S. thypii*, *K. pneumonia*) with zones of inhibition of (6.0 mm and 13.0 mm) and (12.0 mm, 10.0 mm and 13.0 mm) respectively. Also Ni carboxylate inhibited gram positive (*S. faecalis*) and gram negative (*P. fluorescence*, *S. thypii*, and *K.*

pneumonia) at (12.0 mm) and (16.0 mm, 12.0 mm and 10.0 mm) respectively.

However, the derivative of Ni carboxylate was significantly pronounced in the extent of inhibition when compared with that of Cu (Table 2). Ni carboxylate derivative has zone of inhibition of 12.5 mm while Cu carboxylate derivative has 6.5 mm against gram positive organism (*S. faecalis*), also the derivatives of Cu and Ni carboxylates exhibited antimicrobial properties against gram negative (*P. fluorescence* and *S. thypii*). Figures 2, 3 and 4 are clear representations of the zones of inhibition of Cu and Ni derivatives.

Carboxylates of Cu and Ni and their calcinated derivatives showed antimicrobial activity against gram positive and gram negative organisms with a comparable zone of inhibition to that of the standard antibiotic used for the assay. The calcinated derivatives of the carboxylates displayed antibacterial activity against gram negative (*P. fluorescence* and *S. thypii*) (Table2) and this result is similar to the findings of Srisung *et al.* where Ni and Cu complexes were able to elicit antibacterial activity against gram negative organisms (*S. pyogenes* and *P. shigelloides*) and (*S. typhimurium*) respectively. The mechanism of bactericidal action of metallic compounds had been attributed to their ability to release metal ions in the nutrient media and that the media facilitated the release of the metal ion (Ruparelia et al.,2008). The ions released may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death (Lin et al., 1998). Ions inside the bacterial cells may bind to deoxyribonucleic acid molecules and become involved in cross-linking within and between the nucleic acid strands, resulting in the disorganized helical structure. In addition, copper ion uptake by the bacterial cells had also been reported to damage important biochemical processes (Kim et al., 2000; Stohs and Bagchi, 1995). The observed difference in the resistance of both gram-positive and -

negative bacteria populations to the carboxylates and their calcinated derivatives could be attributed to variations in the cell structure, physiology, metabolism, or degree of contact of organisms with the compounds.

Conclusion: Cu and Ni carboxylates of castor seed oil were prepared by metathesis in ethanol and the formation of the carboxylates were confirmed from FTIR spectra. The carboxylates calcinated at 400°C yielded blackish derivatives suspected to be oxides of the metals based on their colour. The results of antimicrobial activities revealed that the carboxylates and their

calcinated derivatives have comparable activities with standard antibiotic. The two carboxylates exhibited similar activities against the tested organism. Cu carboxylate was more effective than its calcinated derivative while the reverse is the case for Ni carboxylate.

The results obtained so far could be a platform for further studies on the size and temperature dependent antimicrobial activities of calcinated derivatives of Cu and Ni carboxylates against some major nosocomial organisms.

Table 1: Antimicrobial effects of Cu and Ni Carboxylates on gram positive and gram negative microorganisms.

Tested Organisms	Zones of inhibition (mm)				
	Standard Antibiotics Ampicillin	Carboxylate Cu Ni		Blank	Acetone
<i>Salmonella typhi</i>	20.0	10.0	12.0	0.0	0.0
<i>Staphylococcus aureus</i>	18.0	6.0	0.0	0.0	0.0
<i>Streptococcus faecalis</i>	20.0	13.0	12.0	0.0	0.0
<i>Pseudomonas fluorescens</i>	21.0	12.0	16.0	0.0	0.0
<i>Klebsiella pneumonia</i>	22.0	13.0	10.0	0.0	0.0

* Each value represents the mean of three repeat experiments.

Table 2: Antimicrobial effects of calcinated derivatives of Cu and Ni carboxylates on some organisms of medical importance

Tested Organisms	Zones of inhibition (mm)				
	Standard Antibiotics Ampicillin	Calcinated Carboxylate Cu Ni		Blank	Acetone
<i>Salmonella typhi</i>	20.0	7.0	14.0	0.0	0.0
<i>Staphylococcus aureus</i>	15.0	0.0	0.0	0.0	0.0
<i>Streptococcus faecalis</i>	20.0	6.5	12.5	0.0	0.0
<i>Pseudomonas fluorescens</i>	15.0	9.0	10.0	0.0	0.0
<i>Proteus mirabilis</i>	14.0	0.0	0.0	0.0	0.0

* Each value represents the mean of three repeat experiments.

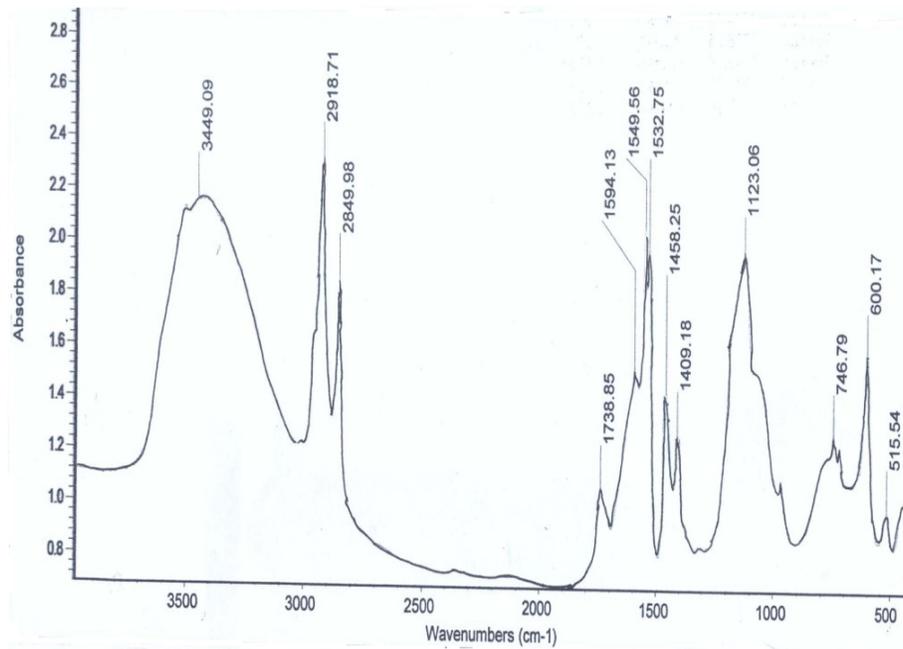


Fig 1: FTIR spectrum of Cu carboxylate of castor seed oil.

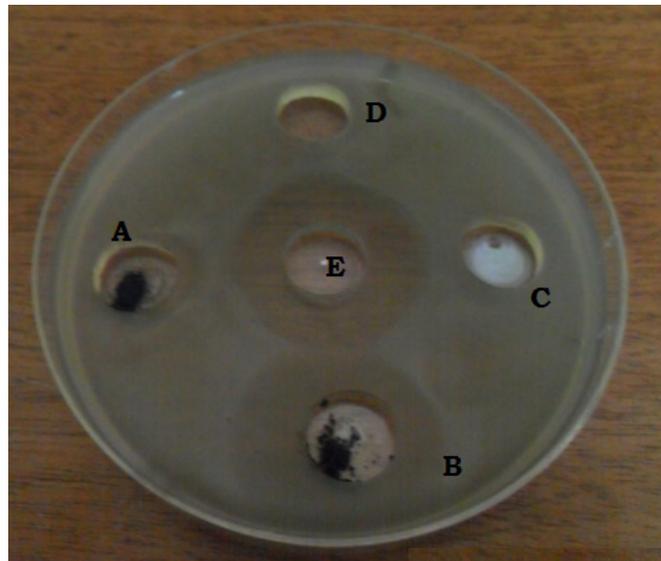


Fig: 2: Inhibition of *Pseudomonas fluorescens* in agar well diffusion assay.

- A – well containing calcinated derivative of Cu carboxylate [100 μ L (50 μ g)]
- B – well containing calcinated derivative of Ni carboxylate [100 μ L (50 μ g)]
- C– well containing acetone (0.1ml)
- D– well containing distilled water (0.1ml) (blank)
- E – well containing standard antibiotics ampicillin [250mg/ml]

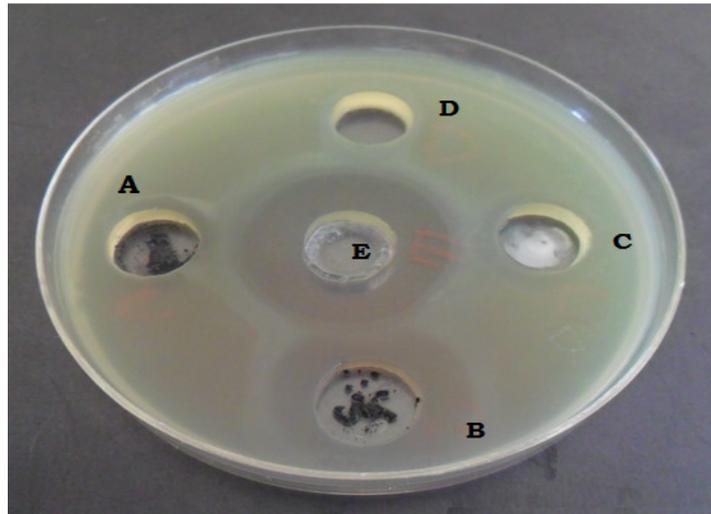


Fig: 3: Inhibition of *Salmonellatyphi* in agar well diffusion assay.

- A – well containing calcinated derivative of Cu carboxylate [100 μ L (50 μ g)]
- B – well containing calcinated derivative of Ni carboxylate [100 μ L (50 μ g)]
- C– well containing acetone (0.1ml)
- D– well containing distilled water (0.1ml) (blank)
- E – well containing standard antibiotics ampicillin [250mg/ml]

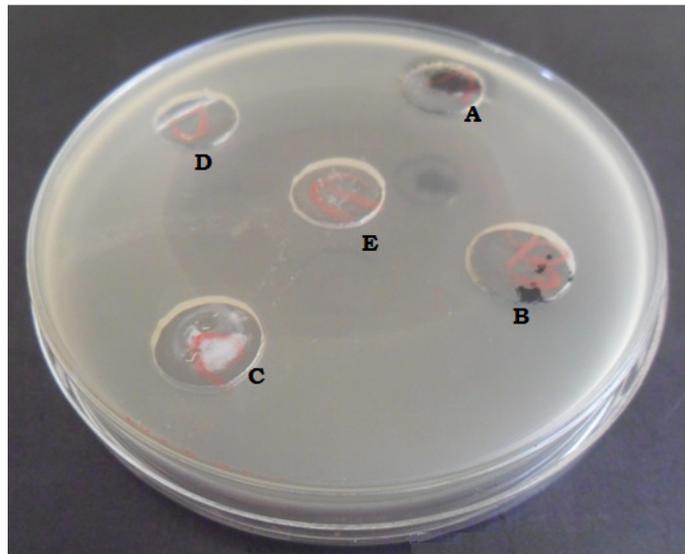


Fig:4: Inhibition of *Streptococcus feacalis* in agar well diffusion assay.

- A – well containing calcinated derivative of Cu carboxylate [100 μ L (50 μ g)]
- B – well containing calcinated derivative of Ni carboxylate [100 μ L (50 μ g)]
- C– well containing acetone (0.1ml)
- D– well containing distilled water (0.1ml) (blank)
- E – well containing standard antibiotics ampicillin [250mg/ml]

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