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Antioxidant evaluation and bio-guided isolation from methanol leaf extract of *Acalypha godseffiana*

A. K. Asekunowo[a,b,c,d], I. S. Okoro[e], S. D. Umoh[e,*], X. Siwe Noundou[f], R. W. M. Kraus[b], O. O. Okoh[c], A. O. T. Ashafa[d], O. T. Asekun[a], O. B. Familoni[a]

[a]*Department of Chemistry, Faculty of Science, University of Lagos, Nigeria*
[b]*Department of Chemistry, Faculty of Science, Rhodes University, South Africa*
[c]*Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice South Africa*
[d]*Department of Plant Science, Faculty of Natural and Agricultural Science, University of the Free State, South Africa*
[e]*Department of Chemistry, Department of Chemistry, Joseph Sarwuan Tarka University, Makurdi, Nigeria*
[f]*Department of Pharmaceutical Sciences, School of Pharmacy, Sefako Makgatho Health Sciences University, Pretoria 0204, South Africa*

Abstract

Acalypha godesffiana is a plant used in conventional medicine for fungal-related illnesses. The plant's extracts were investigated in this study, antioxidant, and antimicrobial studies were conducted. Different models were employed in the antioxidant assay; serial dilution was utilized to determine the minimum inhibitory concentration (MIC). The extract from *A. godseffiana* was purified and characterized by chromatographic and spectroscopic techniques, respectively. Three biologically active compounds, 3, 5-dihdroxylbenzoic acid (1), 3, 4, 5-trihydroxybenzoic acid (2), as well as Di-(2-ethylhexyl) phthalate (3), were identified for the first time in *A. godseffiana*. The extract and fractions exhibited varying scavenging capacities on different anti-oxidative models. The DPPH of MeOH (IC_{50}= 0.51 mg mL^{-1}) was comparable with silymarine (SLY) IC_{50}= 0.52 mg mL^{-1} and better than gallic acid (GAL) IC_{50}=1.95 mg mL^{-1}; the $ABTS^{\cdot+}$ of EtOAc column fraction (ACF, IC_{50}=0.46 mg mL^{-1}) was comparable with standard SLY, IC50 = 0.47 mg mL^{-1}; and an OH radical of DCM, IC_{50}= 0.10 mg mL^{-1}, was better than both standards (SLY, IC_{50}= 6.30 mg mL^{-1}, GAL: IC_{50}= 1.93 mg mL^{-1}). ACF showed superior antifungal activities (0.02 mg mL^{-1}) against *Cryptococcus neoformans and Candida albicans*, compared to ketoconazole (MIC of 0.250 mg mL^{-1}). Compounds (1-3) from *A. godseffina* reportedly displayed antioxidant and other activities. This study validated the antifungal potentials of *A. godseffiana* leaves and identified bioactive compounds. The extracts should be further investigated, and the compounds should be added to the existing library for further investigation of possible leads.

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Keywords: *Acalypha godseffiana*, Spectroscopy, Antioxidant, Antifungal, Bioactive compounds

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1. Introduction

Medicinal plants are crucial in the fight against drug resistance, as they serve as foundations for chemical agents, lead drugs, and new drugs [1, 2]. In 2001, 25% of the world's best-selling medications were natural products or bioinspired medi-

*Corresponding author Tel. No.: +234-703-134-3987.
Email address: sampson.umoh@uam.edu.ng (S. D. Umoh)

cations [3]. This report was evident in that the bark of *Cinchona* is the source of malaria-attacking quinine; opium is a source of codeine, morphine, and paregoric (an anti-diarrhea agent), while morphine is still an acceptable pain-relieving medication to date [4]. Aspirin is made from salicin, a glycoside that was separated from *Salix alba* Linn. Secondary metabolites from plants, such as flavonoids, alkaloids, terpenoids, and tannins, have antibacterial properties, making them a cost-effective alternative to synthetic antibiotics [5]. Plant-derived metabolites, including flavonoids, alkaloids, terpenoids, and tannins, have antibacterial properties, suggesting they could be a cost-effective and secure alternative to antibiotics in treating microbial diseases [6]. Plant metabolites possess antibacterial properties, acting either bacteriostatic or bactericidal on bacterial strains [7]. Novel antibiotic prototypes have been reported to be promising among higher plant sources [8]. Plant phytochemicals, including phenolic compounds, nitrogen-containing compounds, and terpenes, are known to have antifungal properties.

The *Euphorbiaceae* plant *Acalypha godesffiana* MUELL ARG, also known as fire dragon, match-me-if-you-can, and Joseph's coat, is popular in the *Euphorbiaceae* family [9]. It originated from the Pacific Islands and spread to the world, mostly to the tropics of Africa, Asia, and America. In Nigeria, the Yorubas' call it 'Jinwinini'; the Igbos call it "Ogbunizu". Traditional therapies make use of this plant due to its therapeutic properties, which are used to treat illnesses like fungal infections. The antimicrobial potentials of the leaf samples of the plants concerning solvents for extraction have been documented [10]. The phytochemical composition of the plant's parts has been previously documented to include phenols, saponins, alkaloids, cardiac glycosides, terpenoids, and flavonoids [10–12]. *Acalypha* species contain compounds like gallic acid, ethyl gallate, methyl gallate, ellagic acid, brevifolin carboxylate, 1, 2, 3-benzenetriol and kaempferol-3-O-α-L-rhamnoside [13, 14]. In this study, we investigated bioactive constituents, antioxidants, and antibacterial properties of partitioned extracts. This work produced compounds (1-3) from *A. godesffiana* for the first time.

2. Materials and methods

2.1. Plant collection and authentication

Acalypha godseffiana leaves were collected at Eziala Mgbidi, Oru West Local Government Area, Imo State, Eastern Nigeria (Elev 94 N 05° 44° 44.7″, E 006° 54° 097″) between August and December 2014. The plant specimen (LUH 2753) was verified by Dr. A. B. Kadiri of the Department of Botany, Faculty of Science, University of Lagos, Akoka, Nigeria, Herbarium.

2.2. Preparation of plant extract

The 1.5 kg ground sample of *Acalypha godseffiana* was macerated for 72 hours at room temperature in a shaker with 20 L of methanol. The solution was filtered, and 245.25 g of crude methanol extract was obtained by a rotary evaporator at 37 °C.

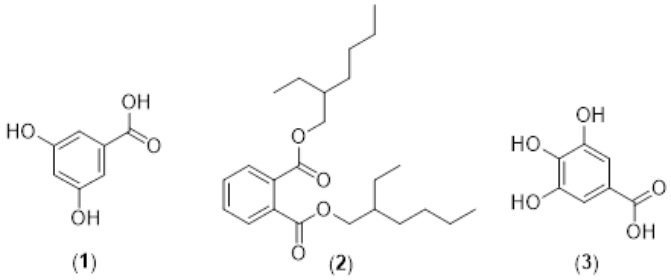

Figure 1. Compounds identified from *Acalypha godseffiana* leaf extract.

2.3. Solvent-solvent fractionation

After being suspended in 100 milliliters of water, the 245.25 grams of crude methanol extract were divided into four parts using n-hexane, dichloromethane, ethyl acetate, and finally n-butanol within that line, except for the extract of n-butanol, which was concentrated using a water bath at 90 °C. Each resultant fraction was independently concentrated by evaporation under a fume hood to give their respective fractions.

2.4. Antioxidant assays

The antioxidant potentials of methanol extract and its partitioned fractions (HEX, DCM, EtOAc, BUOH, and ACF) were determined using six methods: DPPH, TAC, ABTS, FRAP, OH radicals, and MCH assays.

2.4.1. Diphenylpicrylhydrazyl (DPPH) radical scavenging properties

Previously established approach [15] was followed with minor adjustments to quantify the impact of each extract or fraction on DPPH radicals: Each extract or fraction was prepared in five different strengths (0.125–0.1 mg mL^{-1}) and combined with a 1.0 mL methanol-based solution containing 0.135 mM DPPH radical. After shaking the reaction solution and letting it remain at room temperature under dark conditions for thirty minutes, the amount of light it absorbed was measured. with a spectrophotometer set at 517 nm.

2.4.2. Phosphomolybdenum-based total antioxidant capacity (TAC) method

To evaluate the TAC of extracts and fractions based on their capacity to convert Mo (VI) in an acidic medium to Mo (V), previously modified methodology [16] with the ability to transform Mo (VI) into Mo (V) was adopted. In 96-well plates, different quantities of a reagent solution, including ammonium molybdate, sodium phosphate, and sulfuric acid, were mixed with the extracts or reagent mixture, including ammonium molybdate, sulfuric acid, and sodium phosphate fractions. The blank was made by combining 300 μL of test reagent solution with 30 μL of methanol, with silymarin and gallic acid serving as standard controls. Using a microplate reader, measurements were made for the amount of Mo (VI) reduced to Mo (V) after being kept at a temperature (95 °C) for ninety minutes.

Table 1. IC_{50} of leaf extract and fractions of *A. godseffiana* antioxidant potentials.

ASSAY	SLY	GAL	MEOH	HEX	DCM	EtOAc	BuOH	ACF
	IC_{50} (mg mL^{-1})							
DPPH	0.52 ± 0.05^{a}	1.95 ± 0.05^{b}	0.51 ± 0.06^{a}	0.53 ± 0.05^{a}	0.46 ± 0.06^{a}	0.64 ± 0.08^{c}	0.48 ± 0.04^{a}	1.01 ± 0.02^{d}
MCH	3.06 ± 0.05^{a}	1.03 ± 0.03^{b}	0.47 ± 0.06^{c}	0.67 ± 0.05^{c}	0.71 ± 0.04^{c}	5.12 ± 0.05^{d}	5.66 ± 0.06^{e}	6.11 ± 0.05^{f}
FRAP	0.38 ± 0.05^{a}	1.03 ± 0.03^{b}	2.31 ± 0.06^{c}	3.17 ± 0.05^{d}	3.06 ± 0.06^{d}	1.26 ± 0.07^{b}	1.74 ± 0.02^{e}	1.27 ± 0.04^{b}
ABTS	0.47 ± 0.05^{a}	0.96 ± 0.01^{b}	0.55 ± 0.04^{a}	0.54 ± 0.03^{a}	0.59 ± 0.03^{a}	0.57 ± 0.04^{a}	0.58 ± 0.05^{a}	0.46 ± 0.05^{a}
TAC	0.56 ± 0.06^{a}	0.52 ± 0.03^{a}	0.62 ± 0.03^{a}	0.43 ± 0.05^{a}	0.10 ± 0.05^{b}	0.52 ± 0.03^{a}	0.59 ± 0.05^{a}	0.51 ± 0.05^{a}
OH	6.30 ± 0.04^{a}	1.93 ± 0.06^{b}	1.41 ± 0.02^{c}	2.49 ± 0.05^{d}	0.10 ± 0.04^{e}	0.84 ± 0.06^{e}	2.27 ± 0.0^{d}	2.49 ± 0.05^{d}

Key: The IC50 values derived from the linear regression equation are displayed as mean ± SEM (n = 3). Results for each parameter that has distinct superscripts in the same row are statistically significant ($p < 0.05$) with one another. FRAP: ferric reducing ability of plasma; MCH: metal chelating ability; DPPH: 1, 1-Diphenyl-2-picryl hydrazyl The ABTS radical cation is 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate), TAC: total antioxidant capacity by phosphomolybdenum; OH: hydroxyl radical scavenging capacity; SLY: Silymarine and GAL: gallic acid as standard antioxidants. MeOH: Methanol Extract, HEX:Hexane fraction, DCM :Dichloromethane fraction, EtOAc :Ethyl acetate fraction, BUOH: Butanol fraction, ACF: Column Fraction.

Table 2. MIC (mg mL^{-1}) of extract/fraction of *A. godseffiana* (leaves) against human pathogenic bacteria and fungi.

Extract/ Fractions	*C. albicans*	*T. mucoides*	*C. neoformans*	*S. aureus*	*P. aeruginosa*
MeOH	0.098	3.125	0.098	3.125	1.563
HEX	0.059	0.937	0.059	0.938	1.875
DCM	0.469	0.469	0.117	1.875	0.938
EtOAc	0.059	0.059	0.059	0.469	0.469
BuOH	0.234	0.938	0.059	1.875	1.875
ACF	0.02	0.056	0.02	0.156	0.313
Nystatin	0.25	0.5	0.25	-	-
Ketoconazole	0.25	0.25	0.25	-	-
Streptomycin	-	-	-	0.016	0.016
Tetracycline	-	-	-	0.013	0.013

Key: MeOH = Methanol, HEX = Hexane, DCM =Dichloromethane, EtOAc = Ethylacetate, BuOH = Butanol, ACF = *Acalypha* Column Fraction.

2.4.3. 2,2- Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical ($ABTS^{+}$))

The ABTS+ scavenging activities of extracts and fractions were assessed using a previously documented technique [17], with slight modifications as follows: The stock solution of ABTS radical was obtained by dissolving 7 mM ABTS and 38.4 mg of 2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) in 10 cm^3 of distilled water. In contrast, the potassium per sulfate solution was created by dissolving 6.62 mg K_2S2O_8. ABTS were combined in a one-to-one proportion with K_2S2O_8 and left in the dark for a day.

The study used an ABTS reagent that was made by diluting the stock solution of ABTS radical cation using methanol to get an absorbance at 734 nm of 0.7 ± 0.005. The extract or fraction was added at different concentrations (0.125 to 0.1 mg mL^{-1}) to an ABTS+ solution that had been diluted. After being vortexed, the mixture was left to react for seven minutes before measuring its absorbance with a Japanese-made BIORAD model 680 96-well microplate reader at 734 nm. A control was created using a methanol solvent and ABTS solution alone, with silymarin and gallic acid as common antioxidants.

2.4.4. Ferric reducing antioxidant power assay (FRAP)

Acalypha godseffiana leaf extracts' and fractions' power to decrease ferric ions was ascertained by applying an established Müller *et al.* [18] method. After mixing 2.5 mL of phosphate buffer (20 Mm), 1% KFe(CN)6, and extract/fraction concentrations that range between 0.125 mg and 0.1 mg mL^{-1}, allow incubation at 50 °C for 30 minutes for the whole mixture. The mixture was added to 96-well plates along with 2.5 mL of a 10% weight/volume trichloroacetic acid solution and 0.1% (0.5 mL) weight/volume ferric chloride solution. The mixture is then left to stand for 10 minutes. Next, utilizing a microplate reader with 96 wells (BIO-RAD, model 680, Japan), the measurement of absorbance was at 700 nm. Common antioxidants, like silymarin and gallic acid, were used.

2.4.5. Scavenging activity of Hydroxyl radical (OH) by deoxyribose Method

The deoxyribose technique was utilized to evaluate the extracts and fractions' ability to scavenge hydroxyl radicals [19]. The reaction mixture was combined with different extracts or fractions at varying concentrations (125 μL–1000 μL), containing $FeCl_3$, EDTA, H_2O_2, and 2-deoxy-D-ribose. A 20-mM potassium phosphate buffer with a pH of 7.4 was utilized to

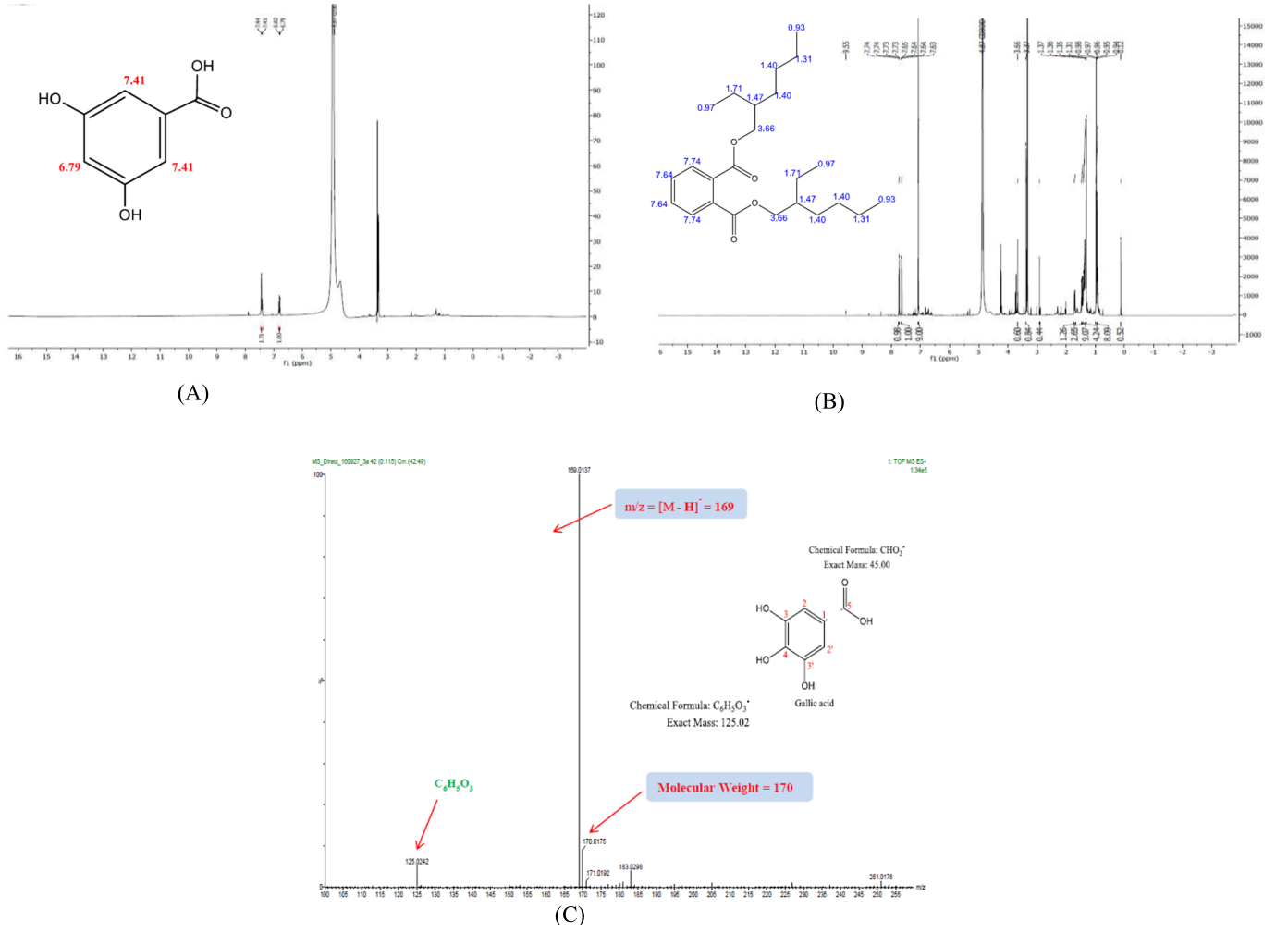

Figure 2. (a) Proton NMR spectrum of compound 1 (b) Proton NMR spectrum of compound 2 (C) HR-ESI-MS spectrum of compound 3; detailed spectroscopic data of compounds 1-3 have been presented in the supplementary document.

prepare the reaction volume. Upon culture for thirty minutes at 37°C, TBA (0.2 mL, 1% w/v) and TCA (0.2 mL, 15% w/v) had been incorporated into the solutions in 0.25 N HCl. After 30 minutes of heating in a bath of boiling water, the resulting mixture of the reaction was allowed to cool. A 96-well microplate scanner was used to quantify the absorbance of deoxyribose degradation at 532 nm, yielding a percentage inhibition percentage. The reaction volume was made using a buffer solution of potassium phosphate (20 mM, pH 7.4). After 30 minutes of incubation, 15% w/v and 0.2 mL of TCA at 37 °C, as well as 0.2 mL and 1% w/v of TBA, were introduced into the solutions in 0.25 N HCl. After 30 minutes of heating in a bath of boiling water, the blend used for the reaction was allowed to cool. A 96-well microplate scanner was used to quantify the absorbance of deoxyribose degradation at 532 nm, yielding a percentage inhibition percentage.

2.4.6. *Metal chelating activity assay*

With slight modifications, previously published methods [20] were used to assess the extracts' and fractions' ability to chelate ferrous ions or Fe^{2+}. Each extract or fraction (40 μL) with variable concentrations that span between 0.125–0.1 mL was applied to a 0.2 mM iron (III) chloride ($FeCl_3$) solution (200 μL). The addition of 5 mM ferrozine (80 μL) after 30 seconds initiated the process. Divalent iron and ferrozine are combined to generate stable, highly soluble in-water magenta complex species. After 10 minutes at 562 nm and of room temperature incubation, the absorbance was calculated while utilizing a 96-well microplate scanner.

2.5. *Minimum inhibitory concentration (MIC) of extracts/fractions*

The antibacterial potential of MeOH extract, HEX, DCM, EtOAc, BUOH, and ACF fractions was assessed using the minimum inhibitory concentration (MIC) in 96-well microtitre plates in cooperating two-fold serial dilutions of the fractions. The bacteria strains tested were *Staphylococcus aureus and Pseudomonas aeurginosa,* while *the* fungi strains were *Candida. albicans, Trichophyton mucoides,* and *Cryptococcus. neoformans*. The strains grew in a nutritional broth that had been autoclaved for 24 hours for fungus and 18–24 hours

for bacteria, then corrected to the last level of density of 106 cfu/mL. The strains were cultivated to an ultimate density of 106 cfu/mL in autoclaved nutrient broth. After 24 hours of aerobic incubation (25 °C for fungus and 37 °C for bacteria), the plates were examined.

To quantify the amount of microbial growth, 40 microliters of 0.2 milligrams per milliliter of p-iodonitrotetrazolium solution (purity 97%, Sigma, South Africa) were introduced into every well. The wells were subsequently incubated for thirty minutes at 25 °C for fungi and 37 °C for bacteria. The organisms' activity converted the colorless tetrazolium salt to a reddish-brown result. Every treatment was performed in triplicate, and for a therapy to be deemed active, growth had to be completely suppressed at a particular fraction concentration [21].

2.6. Bioassay guided isolation of bioactive compounds

Further purification of the EtOAc fraction of MeOH extract—the most antifungal fraction—was achieved employing preparative thin-layered chromatography (PTLC) and frequent column chromatography. Fraction C from the first column was re-crystallized using hexane, chloroform, and ethyl acetate to afford compound 1. The third column's fraction, DB1L, was purified using preparative thin layer chromatography (PTLC) using a 5:4:1 ratio of chloroform, ethyl acetate, and formic acid to produce compounds 2 and 3.

2.7. Structural elucidation of the isolated compounds

Spectroscopic methods (NMR, ESI-MS, and FT-IR) were employed to elucidate the structure of the compounds. Utilizing as an internal standard tetramethylsilane (TMS) with CD_3OD and $CDCl_3$, at standard room temperature, a Bruker Avance II 600 MHz Fourier transform NMR spectrometer was used to obtain the nuclear magnetic resonance spectroscopic data. Water Synapt G2 mass spectrometry was used to record high-resolution mass spectra. A Bruker FT-IR spectrometer was employed to obtain the infrared spectra of neatly isolated compounds. The melting point was determined using mercury in a glass thermometer and an electrothermal IA 9300 capillary melting point device.

2.8. Statistical analysis

The study used GraphPad Prism 5 for data analysis, including one-way ANOVA, Bonferroni tests, and student t-tests carried out at a p-value of less than 0.05.

3. Results and discussion

3.1. Antioxidant capacities

The fractions' and extracts' antioxidant capacities were also accessed using their IC_{50} values (Table 1). The best scavenging capacity was exhibited by the DCM fraction on DPPH (IC_{50}= 0.46 mg mL^{-1}), comparable with SLY's IC_{50} = 0.52 mg mL^{-1} and better than GAL's IC_{50}= 1.95 mg mL^{-1}), on OH radicals (IC_{50}= 0.10 mg mL^{-1} was better than both standards (SLY, IC_{50}= 6.30 mg mL^{-1}, GAL, IC_{50}= 1.93 mg mL^{-1}) as well as total antioxidant capacity. TAC of IC_{50} = 0.10 mg mL^{-1}, which was better than both standards (SLY, IC_{50} = 6.30 mg mL^{-1}, GAL, IC_{50} = 1.93 mg mL^{-1}). The best-reducing capacity, FRAP, was exhibited by EtOAc fraction (IC_{50} = 1.26 mg mL^{-1}) and ACF (IC_{50} = 1.27 mg mL^{-1}) and was comparable with GAL (IC_{50} = 1.03 mg mL^{-1}). Metal chelating capacity, MCH of the MeOH, IC_{50}= 0.47 mg mL^{-1}, was significantly equivalent to that of HEX (IC_{50}= 0.67 mg mL^{-1}) and DCM (IC_{50}= 0.71 mg mL^{-1}) fractions and better than (SLY, IC_{50}= 3.06 mg mL^{-1}, GAL, IC_{50}= 1.03 mg mL^{-1}). ACF (IC_{50} = 0.46 mg mL^{-1}) exhibited a comparable scavenging capacity with SLY (IC_{50} = 0.47 mg mL^{-1}) on ABTS radicals.

Antioxidants are necessary to control oxidative reactions in human cells and, thus, prevent harm or even cell death. This investigation demonstrated that ABTS.+ had better scavenging properties by ACF (IC50, ACF = 0.46 mg mL^{-1}) than did DPPH (IC50, ACF = 1.01 mg mL^{-1}), corroborating previous research [4] that suggested certain compounds with ABTS.+ scavenging activity were unable to scavenge DPPH. Similar to what was discovered and described, certain compounds are moderate DPPH as well as ABTS.+ scavengers. This supports the notion that the extracts and fractions have varying capacities for scavenging free radicals, a helpful strategy for addressing pathologic damage related to free radicals [22]. A FRAP assay was also employed to assess the extracts' or fractions' antioxidant capacities. It has been previously established that the FRAP assay will detect most secondary metabolites that are redox-active substances [23]. The analysis's findings proved compliant with the literature report of Sowndhararajan & Kang [24], showing that the extracts had moderate reducing capacity (FRAP), except the EtOAc fraction and ACF, which showed the best activity at the respective IC_{50s} of 1.26 mg mL^{-1} as well as 1.27 mg mL^{-1}. The phosphomolybdenum approach, which relies on a transformation of Mo (VI) to Mo (V) through antioxidant compounds as well as the generation of phosphate Mo (V) complexes with a green colour, was used to assess the TAC of the extracts or fractions.

According to this methodology, greater antioxidant activity is correlated with higher TAC values [25, 26]. This study's findings demonstrated that DCM fractions exhibited better activity while other fractions had comparable phosphomolybdenum reduction capacity to the standards. This study's TAC outcome is consistent with earlier research [27]. The extracts' discovered antioxidant properties were linked to their redox features in controlling harmful reactive oxygen species (ROS) chain reactions and absorbing and eliminating free radicals that possess a preventative effect on the formation of malignancies. The high concentration of flavonoids, proanthocyanidins, and polyphenols found in *A. godseffiana* leaves may be the cause of the extracts' and fractions' potent antioxidant activity.

3.1.1. Antimicrobial activities

The outcomes of the antibacterial examination showed that the fraction of ethyl acetate demonstrated the greatest inhibition against both harmful bacterial strains, *S. aureus* and *P. aeruginosa*, with MIC values of 0.469 mg/mL. Furthermore, with MICs (0.156 mg mL^{-1} and 0.313 mg mL^{-1}) for *S. aureus* as

well as *P. aeruginosa*, respectively, ACF from the ethyl acetate fraction was found to have greater potential against the bacteria tested than both the fractions from methanol and ethyl acetate extracts.

Table 2 presents the specific results in detail. The EtOAc fraction demonstrated robust activity against the three pathogenic fungi tested, namely *Candida albicans*, *Candida mucoides*, and *Candida neoformans*, possessing a uniform MIC of 0.059 mg mL^{-1} superior to the standard antifungal drug, nystatin, which had MICs (0.500 mg mL^{-1}, 0.250 mg mL^{-1} 0.250 mg mL^{-1}) for *Candida mucoides*, *Candida albicans*, and *Candida neoformans*, respectively. Conversely, ketoconazole demonstrated MICs of 0.250 mg mL^{-1} for each of the three (3) tested fungal strains. Compared to the conventional medications, MeOH extract and ACF displayed much superior activity, with MIC values of 0.020 mg mL^{-1} for *Candida albicans*, *Candida neoformans*, and *T. mucoides*, and as indicated in Table 2, this activity may be related to synergistic effects.

Saponins exhibit properties that include antiinflammatory, antimelanogenic, and antispasmodic activities, along with cancer-inhibiting potentials[28]. Saponins, alkaloids, and polyphenolic metabolites are perhaps accountable for the antibacterial characteristics of the *A. godseffiana* plant. Flavonoids are reported to be produced by plants to defend themselves against microbial infections [29]. According to in vitro investigations, flavonoids have been shown to exhibit broad-spectrum antibacterial capabilities [30]. This activity may be related to flavonoids' ability to bind with soluble and extracellular both bacteria's cell walls and proteins. The lipophilicity of flavonoids correlates positively to their ability to disrupt the microbial membrane, which enhances their microbial potential [31].

This study also revealed that, possessing a MIC (0.059 mg mL^{-1}), the EtOAc fraction outperformed the MeOH extract, which had a MIC (0.098 mg mL^{-1}) against the fungal strains *Trichophyton mucoides*, *Candida albicans*, and *Cryptococcus neoformans*. This may be due to the nature of the phytochemicals that are more expressive in EtOAc solvent fractions as compared to MeOH solvent fractions. The antifungal result further revealed that ACF showed better antifungal potency against *C. neoformans* and *C. albicans* (MIC: 0.02 mg mL^{-1}; Table 2), indicating that the extracts express their antifungal potentials less when together; this may arise from antagonistic effects of various components of the extract or fractions.

3.2. Spectroscopic and spectrometric analysis of the isolated compounds

From the ACF fraction, three chemicals were later separated, and their structures were clarified. Compound 1 was obtained from the recrystallization of fraction C in the first column. This was isolated as an off-white crystal (3.0 mg), with a melting point of -238 ^{o}C and a R_f value of 0.37 (Hexane: EtOAc 4:1). The HR-ESI-MS [M-H] = 153.0188 (Calc. 153.0266) corresponds to a molecular formula of $C_7H_6O_4$ (Figure 1). The IR absorption bands show the presence of OH (3188 cm^{-1}) confirming the hydroxyl group, C=C (1598 cm^{-1}) of the aromatic ring, and C=O (1672 cm^{-1}) for carboxylic acid (Figure 1). A tri-substituted benzene ring with two proton signals at δH 6.80 (1H, d, J = 12 Hz) and δH 7.42 (1H, d, J = 18 Hz) was identified in the 1H NMR spectra (CD_3OD, 600 MHz) (Figure 1).

The spectrum of ^{13}C-NMR of compound 1 revealed an aromatic ring with quaternary carbon at positions C1 (δ123.9), C3/C3' (δ146.0), C5 (δ151.2), methine carbons at positions C2/C2' (117.8), and C4 (δ115.7), while the resonance at δ151.2 suggested carboxylic carbon at C5 (Figure 1). The methine carbons were further confirmed with the aid of DEPT-135. Compound 1 was further confirmed to be 3,5-dihydroxybenzoic acid (Figures 1, 2 and supplementary data) with the aid of the 1 and 2-dimensional NMR data, and by comparison with Sowndhararajan & Kang[24].

3,5-dihydroxybenzoic acid, also known as α-resorcylic acid, is being reported here for the very first time, as far as we know, from *Acalypha godseffiana*. It is a polyphenolic acid that may present some natural products that may have healing benefits [32]. It demonstrates the moderate antioxidant and scavenging potential of free radicals and additionally inhibits lipolysis in adipocytes [33].

Compound 2 was obtained from fraction DB_1L of the third column fraction. It emerged as a yellow oily compound (7.0 mg) with a R_f value of 0.61 (Chloroform: EtOAc: Formic Acid 5:4:1). It was elucidated using data obtained from spectroscopic techniques, and the molecular formula was deduced as $C_{24}H_{38}O_4$ through HR-ESI-MS $[M + H]^+$ = 391.2863 (M^+) (Calc. 391.2770). The infrared spectra showed that there was an aliphatic C-H at 2925–2864 cm^{-1}, an aromatic C=C at 1593–1458 1458 cm^{-1}, as well as a carbonyl ester at 1727 cm^{-1}. The ^{1}H-NMR (600 MHz, CD_3OD) showed ortho-substituted aromatic protons at δ_H 7.74 (6 Hz) and δ_H 7.64 (12 Hz).

The proton signal at δ_H 3.68 (2H, m) germinal to the ester carbonyl group was allocated to a methylene group. The methine proton signal was seen at δ_H 1.47 (1H, m), and there were four methylene groups with signals at δ_H 1.31, 1.40, and 1.71 (2H, m). Compound 2's 13C-NMR spectrum showed the anticipated 12-carbon resonance of the molecule (Figure 1). DEPT-135 and HSQC spectra confirmed two quaternary carbons, including the ester carbonyl group at δ 170.4, three methine signals ascribed to aromatic rings at δ 132.9, 132.0, and 128.0, five methylene carbons, including an oxygen-bearing group at δ 170.4 (C4), and two terminal methyl groups (Figures 1, 2 and supplementary data). Compound 2's identity as di-(2-ethylhexyl) phthalate (Figure 1) was verified by comparing the ^{13}C-NMR and 1H values matching those documented in Nair *et al.* [33] and utilizing the COSY and HMBC signals, which correlate to the report of Nair *et al.* [33]. The well-known synthetic plasticizer di-(2-ethylhexyl) phthalate (DEPH) is obtained from a variety of plant species, particularly *Alchornea cordifolia* and *Aloe vera* [34]. Hence, it may have taxonomic significance. The antiviral, anti-tumor, and antioxidant activities of DEHP have been documented [34]. The antimicrobial properties of DEHP were also reported in Abri & Maleki [35].

Further purification of DB_1L using PTLC offered compound 3 as a crystalline colorless powder with a melting

point of -237 ^{o}C and a molecular formula deduced as $C_7H_6O_5$ through HR-ESI-MS [M-H] =169.0167 (Calc. 169.0215). The IR absorption bands show a very broad signal typical of OH at 3199 cm^{-1}, a C=O sharp stretching band at 1672 cm^{-1}, and a C=C aromatic vibration (1598–1437 cm^{-1}) typical of the benzene ring. ^{13}C-NMR signals shown at δ_C 169.32 C=O; 133.57, 132.39, 129.85, 109.68 and ^{1}H-NMR signals at δ_H 7.63, 3.75, 2.35, 1.30 agreed with the literature reports confirming compound 3 (Figure 1, 2 and supplementary data) to be gallic acid [36, 37] previously reported in the literature.

3, 4, 5-trihydroxy benzoic acid, occasionally referred to as gallic acid, is a phenolic acid and has been isolated from species of *Acalypha* [13]. Gallic acid possesses a broad range of actions, according to descriptions, like strong antioxidants, scavenging, and antifungal activities of free radicals. The activity of the polyphenolic compounds is influenced by the number and configuration of hydroxyl groups, as evidenced by the fact that, due to the presence of a pair of hydroxyl groups bonded in a meta position to one another in 3,5-dihydroxybenzoic acid (α-resorcylic acid), gallic acid has higher biological activities [32]. One of *Toona sinensis*' main ingredients, gallic acid, has been suggested as a possible medication agent to treat prostate cancer [38].

4. Conclusion

This study investigates the antioxidant and antimicrobial properties of *Acalypha godesffiana*, a plant used in conventional medicine for fungal-related illnesses. The extracts were purified and characterized using chromatographic and spectroscopic techniques. Three biologically active compounds were identified, including 3, 5-dihdroxylbenzoic acid (1), 3, 4, 5-trihydroxybenzoic acid (2), and Di-(2-ethylhexyl) phthalate (3). The extract and fractions showed varying scavenging capacities on different anti-oxidative models. The study validates the antifungal potentials of *A. godseffiana* leaves. The phytochemicals and antioxidant activities revealed in this study can be employed for human health benefits.

This study's biological activities support the plant's use in folk medicine as a potent antifungal therapy for fungal skin infections. Many of the biological activities reported in the literature for the compounds correlate well with the activities revealed in this study, suggesting that the three isolated compounds may be responsible for the potent antifungal capacity exhibited by the EtOAc fraction and column fraction (ACF) of *Acalypha godseffiana* methanol leaf extract from this study. Further investigations are needed to fully explore the biological potentials of *Acalypha godseffiana.*

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