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Isolation, Identification and Biological Activities of the Constituents of *Hibiscus rosa-sinensis* Leaf Extract

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Abstract
Hibiscus rosa-sinensis has over the years been used in the treatment of bacterial infection and oxidative stress related diseases. In this study, the leaf extract of *Hibiscus rosa-sinensis* (Linn) was investigated for total phenolic content, total flavonoid content, antioxidative potential, acetylcholinesterase inhibitory and antibacterial activities. *In vitro* bioactivity-guided isolation led to the isolation of compound **1** and a mixture of compounds **2** and **3** from the aqueous methanol fraction. The extract, fractions and isolated compounds were investigated for their antioxidant, acetylcholinesterase inhibitory and antibacterial activities. The crude methanolic extract has the highest phenolic constituents TPC and TFC (86.14±1.67 mg GAE/g and 10.25±7.89 mg QUE/g) respectively. The crude methanol extract also exhibited a higher ferric reducing antioxidant power (FRAP) than the aqueous methanol fraction with a value of 42.19±1.89 mg AAE/g. Mixture of compounds **2** and **3** has the highest total antioxidant capacity TAC (36.06±1.09 mg AAE/g), DPPH free radical scavenging activity (0.09±0.05 mg/mL) and acetylcholinesterase inhibitory AChEI (0.86±0.13 mg/mL) in IC_{50} activities. The crude extract and compound **1** showed considerable antibacterial activity against Gram+ve strain; *Bacillus stereomophillus* and Gram-ve strain; *Escherichia coli* respectively. The isolated compounds were identified using two spectroscopic techniques (IR and NMR). Compound **1** was identified as sucrose, compounds **2** and **3** were identified as scutellarein-6-O-rhamanoside-8-C-glucoside and apigenin-6-C-glucopyranose respectively. This study concluded that crude extract, fractions and isolated compounds showed varying degrees of biological activities ranging from moderate to moderately high activities.

Keywords: *Hibiscus Rosa-Sinensis*, Sucrose, Antibacterial, Spectroscopy,

Introduction

Hibiscus rosa-sinensis (Linn) belongs to the genus Hibiscus (Akpan 2007). It is a shrub widely cultivated in the tropics as an ornamental plant because of their ability to show flower of various colours (Jadhav *et al.*, 2009; Pekamwar *et al.*, 2013; Khristi and Patel 2017). Edible parts of the plant include; flower petals and young leaves (Lim, 2014). Extracts from various parts of the plants (flower, leaf and stem) are used widely in folk medicine in the treatment of a wide range of health conditions including microbial, inflammatory, loss of hair, stomach ulcer, contraceptives and oxidative stress (Prasanna, 2017 and Al-snafi, 2018). Phytochemical screening of the crude extract showed the presence of saponins, flavonoids, tannins, glycosides, phenols, alkaloids resins, phlobatannins and steroids (Garg *et al.*, 2012; Afiffy and Hassan, 2016 and Al-snafi, 2018). Phenolic compounds have been previously isolated from the flower of *Hibiscus rosa-sinensis*, which include: apigenin; vitexin; quercetin-7-O-galactoside; gallic acid; *p*-hydrobenzoic; acid; neochlorogenic acid; scutellarin-6-O-α-L-rhamanophyrsnoside-8-C-β-D-glucopyranoside and kaempferol-7-O-(-6-O-p-hydrobenzoyl gentiobioside) (Salib *et al.*, 2011).

Plant derived antioxidant compounds have earned a lot of trust in its use to protect mankind from oxidative stress damage. It has also been reported that the use of antioxidants may slow down the progression of Alzheimer's disease (AD) and reduce neuronal degeneration (Ertas *et al.*, 2009). Thus, this study investigated the antioxidant, acetylcholinesterase inhibitory and antibacterial activities of *H. rosa-sinensis*.

Materials and Methods

Generals

All solvents used were distilled to ensure purity (*n*-hexane, ethyl acetate, methanol, dichloromethane). Folin-Ciocalteu reagents and ascorbic acid were purchased from Merck, South Africa. 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, and gallic acid were purchased from Sigma Aldrich, Germany. HPLC grade solvents were used in the analysis. All chemicals used were of analytical grade. Aluminum pre-coated TLC Silica gel plates 60 F_{254} were purchased from Merck, Germany.

Analysis of the samples (extract fractions and isolated compounds) was carried out with thin layer chromatography at room temperature using pre-coated plates. Detection of spots was by ultraviolet (UV) lamp (254 and 365 nm). Silica gel was used as the stationary phase for column chromatographic separation. Spectroscopic data were obtained from NMR Bruker; 300 MHz (^{1}H NMR), 75 MHz (^{13}C-NMR) and 400MHz (^{1}H NMR), 100 MHz (^{13}C NMR) respectively. The IR data were obtained from Shimadzu (IR) spectrometer. *In-vitro* biological activities were obtained using UV Spectrophotometer.

Plant Material

Leaves of *Hibiscus rosa-sinensis* were collected within the premises of Obafemi Awolowo

University (O.A.U) Ile-Ife, Nigeria. Mr. Ogunlowo, the curator, Faculty of Pharmacy O.A.U Ile-Ife herbarium, authenticated the plant material and voucher specimen (FPI/2259) was deposited. The leaves were air dried at room temperature and pulverized.

Extraction

Pulverized leaves (1.5 kg) were extracted in a 10 L extraction jar with 100% methanol (8.5 L) at room temperature with occasional shaking until 72 hrs. The extract was filtered and concentrated to dryness using a rotary evaporator at 40 °C. This procedure was repeated continuously until exhaustion. This yielded the methanol crude extract (316 g, 21.06%).

Phytochemical Screening

The crude extract (5 g) was screened to determine the Phytochemicals present in the plant using the methods described by Abulude *et al.,* (2001) as modified by Abulude (2007).

Solvent Partitioning of the Crude Methanol Extract

Crude methanol extract (216 g) was dissolved in distilled water and partitioned with n-hexane (3 x 800 mL). This yielded the aqueous methanol fraction (defatted), (154 g) and the n-hexane fraction (25.45 g).

Total Phenolic and Flavonoid Content (TPC and TFC)

The total phenolic and total flavonoid content were determined using the method developed by Singleton and Rossi (1965) as modified by Gülçin, *et al.* (2004) using the Folin-Ciocalteu phenol reagent for TPC and the aluminum chloride colorimetric assay method as described by Zhishen *et al.* (1999) respectively.

Isolation of Antioxidant Compounds from the Aqueous Methanolic Fraction

Column chromatography using silica gel (70-30 mesh) as the stationary phase was used to fractionate the aqueous methanolic fraction (154 g). The column was eluted gradiently with *n*-Hexane/ethyl acetate/methanol. Flask fractions (250 mL) collected were analyzed on TLC plates using appropriate solvent system (ethyl acetate:methanol). This gave fifteen fractions (HRS A1-HRS C5).

In sub-fractions C1-C5 crystals were formed, which were filtered and washed with methanol. It was further purified by recrystallization, yielding a cream coloured crystalline solids. This was examined on TLC using ethyl acetate:methanol (6.5:3.5) as the solvent system. A single spot was observed and this gave compound **1**. In sub-fractions B1-B5 gave a single spot on analyzing with TLC using ethyl acetate:methanol (8:2). It appeared as greenish yellow viscous liquid after concentration. This yielded a mixture of compounds **2** and **3**.

Quantitative antioxidant activity

Hibiscus rosa-sinensis crude methanol extract, fractions and isolated compounds were

assayed for antioxidant activity using three antioxidant assays (TAC, FRAP and DPPH). In addition, the Total flavonoid and phenolic content were also evaluated.

Total Antioxidant Capacity (TAC)

Hibiscus rosa-sinensis crude extract, fractions and isolated compounds were evaluated for total antioxidant as described by Prieto *et al.,* (1999). It is based on the ability of the sample to reduce Molybdenum (VI) to Molybdenum (V) and the subsequent formation of a green phosphate/Molybdenum (V) complex under acidic medium.

DPPH Free Radical Scavenging Ability

The extract, fractions and isolated compounds from *Hibiscus rosa-sinensis* were evaluated for DPPH free radical scavenging ability as described by Brand-Williams *et al.* (1995). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction (Blois, 1958). The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm.

Ferric Reducing Antioxidant Power (FRAP)

The extract, fractions and isolated compounds from *Hibiscus rosa-sinensis* were evaluated for the ferric reducing antioxidant power FRAP as described by Benzie and Strain, (1996).

Acetylcholinesterase Inhibitory Activity (AChEi)

In-vitro acetylcholinesterase activity of the extract, fractions and isolated compounds of *Hibiscus rosa-sinensis* was evaluated using a modified method of Ellman *et al.,* (1961) as described by Olawuni *et al.,* (2018).

Antibacterial Activity

The samples were subjected to antibacterial sensitivity test using agar-well diffusion method as described by Adegoke *et al.,* 2010.

Results and Discussions

Hibiscus rosa-sinensis has many biological applications because of the presence of bioactive constituents in it. The leaf crude methanolic extract of *H. rosa-sinensis* showed the presence of secondary metabolites such as tannins, resins, saponins, flavonoids, phenols, carbohydrates and alkaloids while phlobatannis, steroids glycosides and terpenoids were found to be absent. This is in agreement with work carried out by Garg *et al.,* (2012), Afify and Hassan (2016) and Al-Snifi (2018) on *H. rosa-sinensis* although this present study showed the presence of tannins (Table1).

Table 1: Phytochemical Screening of Methanolic Crude Extract of *Hibiscus rosa-sinensis*

PHYTOCHEMICALS	ABUNDANCE
TANNINS	+
GLYCOSIDES	-
RESINS	+
SAPONINS	+
PHLOBATANNIS	-
FLAVONOIDS	+
STEROIDS	-
PHENOLS	+
CARBOHYDRATE	+
ALKALOIDS	+
TERPENOIDS	-

Key: + = Presence of Constituents
- = Absence of Constituents

Total Phenolic and Flavonoid Contents

These two parameters are not antioxidant assay but serve as a measure of the presence of phenolics or flavonoids present in a plant extract, which will ultimately reveal the antioxidant potential of the plant. Phenolics and flavonoids are the two classes of plant phenolics which cut across all plants (Cartea *et al.*, 2010). Phenolics are good electron donors because their hydroxyl group can directly contribute to the total antioxidant capacity (Aryal *et al* 2019). The crude methanol extract and the fractions were evaluated for TPC and TFC. Crude methanol extract has the highest phenolic constituents for both TPC and TFC expressed as 86.14±1.67 mg GAE/g and 10.25±7.89 mg QUE/g respectively. The aqueous methanolic fraction also followed with 83.03±0.77 mg GAE/g and 8.91±0.59 mg QUE/g while n-hexane fraction has the least phenolic and flavonoid constituents for TPC and TFC (Table 2).

Table 2: Total Phenolic and Flavonoid Content in Crude Methanolic Extract and Solvent Fractions of *Hibiscus rosa-sinensis*

CONSTITUENTS	Total Phenolic Content (TPC) in mg GAE/g	Total Flavonoid Content (TFC) in mg QUE/g

Crude Methanol Extract	86.14±1.67	10.25±7.89
Aq. Methanol Fraction	83.03±0.77	8.91±0.59
n-Hexane Fraction	53.22±2.40	2.78±0.07

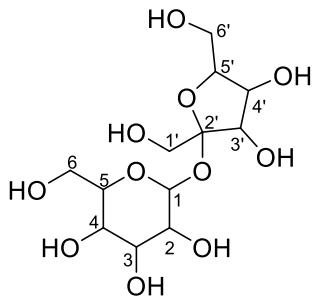
Data are expressed as mean ± SD in three replicates

Structural Elucidation of Isolated Compounds

Isolation of compounds from the aqueous methanol fraction of *Hibiscus rosa-sinensis* was carried out by bioactivity-guided fractionation to isolate antioxidant, acetylcholinesterase inhibitory and antibacterial constituents of the of *Hibiscus rosa-sinensis* leaf extract. Three compounds (1-3) were isolated and characterized using IR and 1D-NMR (^{1}H NMR, ^{13}C NMR, DEPT-135) (Table 3-4).

Characterization of Compound 1

Compound **1** was obtained as a cream coloured crystalline solids. The IR spectrum showed the following absorption bands O-H (3389.04 cm^{-1}), sp^3 C-H (2941.51cm^{-1}), C-O (1068.60 cm^{-1}). The ^{1}H–NMR DMSO-d_6 (300Hz) of compound **1** showed 12 peaks between δ 3.46-5.20 ppm which were non-aromatic protons but are characteristic of sugar moiety. The spectrum showed peaks at the following chemical shifts δ_H 5.20 ppm (H-1), 3.61 ppm (H-2), 3.65 ppm (H-3), 3.46 ppm (H-4), 3.58 ppm (H-5), 3.75 ppm (H_a-6 and H_b-6), 3.66 ppm (H-1′), 4.37 ppm (H-3′), 3.90 ppm (H-4′), 3.87 ppm (H -5′), 3.77 ppm (H-6′). The ^{13}C-NMR (75Hz, DMSO-d_6) spectrum gave 12 peaks between δ_C 60.9-104.0 ppm. The spectrum showed peaks at the following chemical shifts which were assigned to the following carbon atoms with δ_C 92.1 ppm (C-1), 72.0 ppm (C-2), 73.3 ppm (C-3), 70.2 ppm (C-4), 73.2 ppm (C-5), 60.9 ppm (C-6), 62.4 ppm (C-1′), 104.1 ppm (C-2′), 77.4 ppm (C-3′), 74.7 ppm (C-4′), 82.9 ppm (C-5′), 62.5 ppm (C-6′). DEPT-135 Spectrum also revealed the presence of 8 methine (CH) and 3 methylene (CH_2) groups. Also observed is the presence of chemical shifts δ_C 104.1 due to a quartenary carbon C-2'. Compound **1** was identified as sucrose, a disaccharide sugar from spectroscopic data which were in agreement with the literature (Jones *et al.*, 1979) (Table 3).



(1)

Figure 1: Chemical Structure of Sucrose Isolated Compound**;** from the extract of *Hibiscus rosa-sinensis*

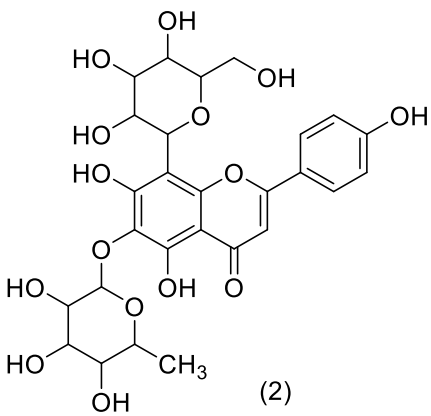
Table 3: Comparison of NMR Spectroscopic Data (300 MHz for ^{1}H and 75 MHz for ^{13}C) of Isolated compound **1** (HRS-C1) with literature values of Sucrose

Positions	**^{1}H-NMR Data (ppm)**		**^{13}C-NMR Data (ppm)**	
	Compound **1** (DMSO-d_6)	Literature	Compound **1** (DMSO-d_6)	Literature
1	5.20	5.45	92.1	92.9
2	3.61	3.65	72.0	71.9
3	3.65	3.77	73.3	73.4
4	3.46	4.47	70.2	70.0
5	3.85	3.86	73.2	73.2
6	3.75	3.83	60.9	61.0
1′	3.66	3.68	62.4	62.2
2′			104.1	104.5
3′	4.37	4.42	77.4	77.3
4′	3.90	4.05	74.7	74.8
5′	3.87	3.89	82.9	82.2
6′	3.77	3.83	62.5	63.2

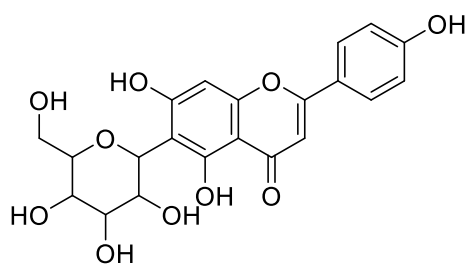
Literature: Jones *et al.* 1979.

Characterization of Mixture of Compounds 2 and 3

The mixture of compounds **2** and **3** was obtained as a greenish yellow viscous liquid. The IR spectrum showed the following absorption bands O-H (3404.47 cm^{-1}), C=C (1654.98 cm^{-1}), sp^3 C-H (2937.68 cm^{-1}), C=O (1724.45 cm^{-1}), C-O (1045.45 cm^{-1}). The mixture components were resolved by comparing the NMR peak intensities. Compound **2** and **3** were assigned to peaks of higher and lower intensities respectively. The ^{1}H –NMR spectrum of both compounds **2** and **3** show signals for both aromatic and sugar moieties. The ^{13}C -NMR spectrum (100Hz, DMSO-d_6) revealed 48 carbon peaks 27 for compound **2** and 21 for compound **3.** Compound **2** was confirmed as a flavonoid with two sugar moieties. The spectroscopic data are in agreement with the literature (Salib *et al.,* 2011), showing that compound **2** was identified as scutellarein-6-O-rhamanoside-8-C-glucoside (2). Compound **3** was confirmed as a flavonoid with one sugar moiety. The spectroscopic data are in agreement with the literature (Gumiaraes *et al.,* 2015) showing that compound **3** was identified as apigenin-6-C-glucoside (isovitexin) **(3)** (Tables 4 and 5).



(2)



(3)

Figure 2: Chemical Structures of Isolated Compound **2** and **3** from the extract of *Hibiscus rosa-sinensis*

Table 4: Comparison of ^{13}C NMR data of compound **2** (HRS-B1) with literature values of Scutellarein-6-O-rhamanoside-8-C-glucoside

Compound **2**	(DMSO-d_6)	Literature (MeOH)
$\mathbf{C_2}$	164.35	164.25
$\mathbf{C_3}$	101.12	102.58
$\mathbf{C_4}$	182.35	182.35

C_5	155.88	158.35
C_6	129.33	131.40
C_7	161.44	160.21
C_8	105.81	104.70
C_9	155.05	152.91
C_{10}	104.75	104.09
C_1'	121.52	121.55
C_2'	129.23	129.26
C_3'	116.08	116.20
C_4'	161.94	162.20
C_5'	116.08	116.20
C_6'	129.27	129.26
C_1''	101.12	99.80
C_2''	70.98	70.60
C_3''	72.50	70.40
C_4''	72.20	72.20
C_5''	68.39	68.60
C_6''	13.51	17.90
C_1'''	72.28	74.00
C_2'''	73.25	74.40
C_3'''	76.78	78.80
C_4'''	70.21	70.50
C_5'''	81.97	81.80
C_6'''	61.21	60.50

Source: Salib *et al*., 2011

Table 5: Comparison of ^{13}C NMR Spectroscopic Data of Compound **3** (HRS-B1) with Literature values of isovitexin

Compound **3**	(DMSO-d_6)	Literature (MeOH)
$\mathbf{C_2}$	164.35	163.70
$\mathbf{C_3}$	101.39	102.70
$\mathbf{C_4}$	182.11	181.90
$\mathbf{C_5}$	155.05	156.20
$\mathbf{C_6}$	105.81	108.90
$\mathbf{C_7}$	164.35	163.60
$\mathbf{C_8}$	92.95	93.70
$\mathbf{C_9}$	161.94	161.20
$\mathbf{C_{10}}$	104.75	103.30
$\mathbf{C_{1'}}$	121.10	121.10
$\mathbf{C_{2'}}$	128.36	128.50
$\mathbf{C_{3'}}$	116.08	116.20
$\mathbf{C_{4'}}$	161.94	160.70
$\mathbf{C_{5'}}$	116.00	116.00
$\mathbf{C_{6'}}$	128.85	128.50
$\mathbf{C_{1''}}$	78.98	78.90
$\mathbf{C_{2''}}$	73.34	73.10
$\mathbf{C_{3''}}$	70.40	70.50
$\mathbf{C_{4''}}$	70.60	70.20
$\mathbf{C_{5''}}$	81.97	81.60
$\mathbf{C_{6''}}$	61.63	61.50

Source: Gumiaraes *et al.,* (2015)

Antioxidant Activities

Three antioxidant assays were used to examine the quantitative antioxidant activities of the extract, fractions and isolated compounds from *Hibiscus rosa-sinensis*: Total antioxidant

capacity (TAC), Ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging. The crude extract exhibited the highest total antioxidant capacity (TAC) measured as 16.01±0.11 mg AAE/g, ferric reducing antioxidant power (FRAP) 42.19±1.89 mg AAE/g and DPPH free radical scavenging with IC_{50} of 3.99±0.42 mg/mL. Compound **1** at the tested concentrations shows no activity for all the three assays that were tested while the mixture of compounds **2** and **3** showed better activity compared to the crude extract and fractions for both total antioxidant capacity (TAC) 36.06±1.09 mg AA/g and DPPH free radical scavenging (DPPH) with IC_{50} 0.009±0.005 mg/mL. However, compounds **2** and **3** displayed lower activity (13.96 ±0.81 mg AA/g) in ferric reducing antioxidant capacity (FRAP) than the crude, and aq. methanol fraction but higher than the n-hexane fraction (2.78±0.07 mg AA/g). In all, n-hexane fraction as expected displayed the least activity in all the antioxidant assays evaluated (Table 6).

Table 6: Antioxidant activity in crude methanolic extract, solvent fractions and isolated from *Hibiscus rosa-sinensis*

CONSTITUENTS	TAC (mg AAE/g)	FRAP (mg AAE/g)	DPPH (IC_{50}) (mg/mL)
Crude Methanol Extract	16.01 ± 0.11	42.19 ± 1.89	3.99 ± 0.42
Aq. Methanol Fraction	15.76 ± 0.00	40.19 ± 1.26	5.34 ± 0.44
n-Hexane Fraction	9.66 ± 4.03	2.78 ± 0.07	12.25 ± 2.35
Compound **1**	NA	NA	NA
Compound **2** and **3**	36.06 ± 1.09	13.96 ± 0.81	0.090 ± 0.050
Ascorbic Acid (Standard)	-	-	0.008 ± 0.00063

Data are expressed as mean ± SD in three replicates

Acetylcholinesterase Inhibitory Activity

The acetylcholinesterase inhibitory activity expressed in IC_{50} value of the crude methanol extract, solvent fractions and isolated compounds (Table 4) showed that the crude has the highest activity with an AChEi IC_{50} value of 0.44±0.67 mg/mL though far below that of the standard, eserine with 0.068±0.02 mg/mL followed by the mixture of compounds **2** and **3** (0.86±0.13 mg/mL) and aq. methanol fraction (1.03±0.44 mg/mL) while n-hexane fraction is the least active (4.10±1.64 mg/mL). It has been reported earlier that plant essential oils and extracts, rich in terpenes, exhibited a moderately strong AChE and BChE inhibitory activity (Ferhat *et al.,* 2017). Isolated compounds **2** and **3** exhibited IC_{50} of 0.86±0.13 mg/mL. Compound **1** at the tested concentration displayed no activity (Table 7).

Table 7: Acetylcholinesterase inhibitory activity in extract, solvent fractions and isolated compounds from *Hibiscus rosa-sinensis*

SAMPLES	IC_{50} VALUES (mg/mL)
Crude Methanol Extract	0.44±0.67
Aq. Methanol Fraction	1.03±0.44
n-Hexane Fraction	4.10±1.64
Compound **1**	NA
Compound **2** and **3**	0.86±0.13
Standard (Eserine)	0.068±0.02

Data are expressed as mean ± SD in three replicates

Antibacterial Activity

The crude methanol extract, fractions and isolated compounds were subjected to antibacterial sensitivity test against eight bacteria strains three Gram +ve (*Bacillus stearothermophillus, Staphylococcus aureus and Micrococcus luteus)* and five Gram-ve (*Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli and Serratia marcescens*). It is reported that the pathogenic strains of *E. coli* cause diarrhea, urinary tract infections, and can also be the cause of meningitis in newborns. However, *P. aeruginosa* is an opportunistic pathogen common in wounds, catheters, burns and cystic fibrosis patients while *K. pneumoniae* causes pneumonia (Atewolara-Odule *et al.*, 2020). Also reported is *Bacillus stearothermophillus*, which causes mainly food spoilage, *Staphylococcus aureus*. Leading cause of nosocomial infections, are increasingly resistant to an array of antimicrobial agents like penicillin, gentamicin, tobramycin, amikacin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, vancomycin and *Micrococcus luteus* also considered as an opportunistic pathogen that can be responsible for nosocomial infections. The zone of inhibition was the diameter of the circular region around the agar wells where there was no bacterial growth. The crude extract was only active against one Gram +ve bacteria strain (*Bacillus stearothermophillus*) at the tested concentrations (30, 15 and 7.5) mg/mL and exhibited a zone of inhibition of 13 mm, 11 mm and 11 mm respectively. The *n*-Hexane fraction was active against four bacterial strains two Gram +ve (*Bacillus stearothermophillus* and *Staphylococcus aureus*) and two Gram-ve (*Serratia marcescens* and *Micrococus luteus*),. *B. sterothermophillus* at 15, 7.5 and 3.75 mg/mL with a zone of inhibition of 12 mm, 11 mm and 15 mm respectively. It was also active against *Staphylococcus aureus* at only 15mg/mL with a zone of inhibition of 11 mm. For *Serratia marcescens* the zone of inhibition was 12mm at 15mg/m,. *Micrococus luteus* at 15 mg/mL the zone of inhibition was 12 mm. The Aq. methanol fraction was only active against *B. sterothermophillus* at 15 mg/mL with a zone of inhibition of 12 mm. Compound **1** was active against one Gram-ve bacterial strain (*Escherichia coli*). At the tested concentrations (10 mg/mL, 5 mg/mL and 2.5 mg/mL) the zones of inhibition were 12 mm, 18 mm, 16 mm respectively. This is in accordance with

earlier work done by Arullappan *et al.,* (2009) on the antibacterial activity of the crude extracts of petroleum ether, ethyl acetate and methanol of *Hibiscus rosa-sinensis*., Ruban and Gajalakshimi (2012) on the *in vitro* antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens and Khan *et al.,* (2014) on the antibacterial activities of *Hibiscus rosa-sinensis* flower extract. Mixture **2** and **3** at the tested concentration does not exhibit any activity (Table 8).

Table 8: Antibacterial Sensitivity Testing on the Crude Methanol Extract, Solvent Fractions and Isolated Compounds from *Hibiscus rosa-sinensis*

		Zones of Inhibition (Z I)		
Agent	Organism	Concentration (mg/mL)	Diameter of ZI (mm)	Diameter of ZI for *Streptomycin (mm)
Crude Extract	*Bacillus stearothermophilus* (NCIB-822)	30	13	20
		15	11	20
		7.5	11	20
***n*-Hexane fraction**	*Bacillus stearothermophilus* (NCIB-822)	15	12	20
		7.5	11	20
		3.75	15	20
	Staphylococcus aureus (NCIB-4330)	15	11	18
	Micrococcus luteus (NCIB-196)	7.5	11	21
	Serratia marcescens (NCIB-1377)	3.75	12	21
Aqueous methanol fraction	*Bacillus stearothermophilus* (NCIB-822)	15	12	20
Compound **1**	*Bacillus sterothermophillus*	10	NA	20

Compound **1**	(NCIB-822) *Escherichia coli* (NCIB-86)	10	12	19
Compound **2** and **3**		3	NA	20

* Streptomycin is the standard used for the antibacterial activity

NA = No Activity

Conclusions

In this study, the crude extract, fractions and isolated compounds from the leaf of *H. rosa-sinensis* were investigated for *in vitro* antioxidant (using TAC, FRAP and DPPH radical scavenging assays), antibacterial (tested against strains of Gram +ve and Gram -ve bacteria) and acetylcholinesterase inhibitory (AChEi) properties with varying degree of activities ranging from moderate to moderately high activities. In addition, the total phenolic and flavonoids contents (TPC and TFC) of the crude extract and fractions which serves as an indicator of the presence of phenolic and flavonoids were also evaluated. To the best of our knowledge, this is the first report on the AChE inhibition activities of *H. rosa-sinensis*. Thus, the leaf extract, fractions and isolated compound from *H. rosa-sinensis* could hence, be a potential source of lead agents in the management of oxidative stress related, infectious and neurodegenerative disorder.

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