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Original Research Article

Evaluation of the antimicrobial and anticancer properties of the fruits of *Synsepalum dulcificum* (Sapotaceae)

Sheryar Afzal[1], Appala V Raju[2], Chandramathi Samudi Raju[3], Genevieve Chong Zhen Wuii[2], Olorunfemi A Eseyin[4]*

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, MAHSA University, Selangor, Malaysia, [2]Department of Medicinal Chemistry, Faculty of Pharmacy, MAHSA University, Bandar Saujana Putra, Selangor, [3]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia, [4]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria

For correspondence: **Email: olorunfemieseyin@uniuyo.edu.ng; **Tel:** +234-8065161478*

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Abstract

Purpose: *To evaluate the antimicrobial and anticancer activities of the fruit of Synsepalum dulcificum (Sapotaceae) against six bacterial strains and on two different colorectal cancer cell lines, respectively.*
Methods: *The dried fruit of the plant was extracted in a Soxhlet apparatus successively with petroleum ether and ethanol, and concentrated in a rotary evaporator to obtain petroleum ether and ethanol extract, respectively. Phytochemical screening was done on the two extracts. The antimicrobial effects of the extracts against Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogene, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris were evaluated using agar well diffusion technique while the anticancer effect of the ethanol extract was assessed on HCT-116 and primary colon epithelial (PCE) cell lines by MTT assay.*
Results: *The results indicate that the petroleum ether extract of Synsepalum dulcificum fruits exerted stronger antimicrobial activity than the ethanol extract. The ethanol extract also showed significant anticancer activity ($p < 0.05$). The calculated half-maximal concentration (IC_{50}) of the extract on HCT-116 cells at 24, 48, and 72 h are 14.99, 8.97, and 8.54 µg/mL, respectively, while the IC_{50} of the extract on PCE cell lines at 24, 48, and 72 h are 236.25, 206.09, and 196.72 µg/mL, respectively. The extract was more toxic to cancer cells than to normal cells.*
Conclusion: *The results of this study lend some justification for the use of the fruits of Synsepalum dulcificum as an antibacterial and anticancer agent.*

Keywords: *Synsepalum dulcificum, HCT-116, Primary colon epithelial (PCE) cell line, Antimicrobial activity, Anticancer activity*

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INTRODUCTION

Bacteria have been linked to cancer by the induction of chronic inflammation and production of carcinogenic metabolites [1]. *Helicobacter pylori* infection is an example of inflammatory mechanism of carcinogenesis [2]. Reports on human carcinogenesis by bacterial metabolites are not consistent.

According to WHO (2019) [3], Cancer is a large group of diseases characterized by the growth of

abnormal and uncontrolled growth of cells. It is the second leading cause of death globally, accounting for 9.6 million deaths in 2018. In men, cancer of the lung, prostate, colorectal, stomach and liver are the most common. The most common type of cancer in women are those of the breast, colorectal, lung, cervix and thyroid. Besides, cancer is a growing public problem in the world.

The use of herbal remedies is now being widely embraced in many developed countries. It is the most popular and available in the primary healthcare system [4]. *Synsepalum dulcificum, popularly known as miracle* fruit, *magic* fruit, miraculous or flavor fruit, belongs to the family Sapotaceae. [5.] It is used in Africa to sweeten acidulated maize bread (kankies), palm wine and *pito*, a beer made from fermented grains [6]. Although, many scientific reports are available on the pharmacological importance of the leaves and the stem of the plant, information on the antimicrobial and anticancer activities of the fruit of *Synsepalum dulcificums* is scanty. This study aims to evaluate the antimicrobial and anticancer activities of the fruit of the plant.

EXPERIMENTAL

Collection, authentication of plant material, and preparation of plant extracts

Synsepalum dulcificum was bought from Inanam, Kota Kinabalu, Sabah, Malaysia in October 2019. It was authenticated by Mr Aidil, a botanist at Forest Research Institute of Malaysia (FRIM), Kepong, Selangor Darul Ehsan, Malaysia, and given voucher number of "PID 381118-31". A specimen of the plant was deposited in the same Institute.

The fruits of the plant were air-dried under shade, seeds were separated and the pulp, dried, and powdered. The powdered material (100 g) was extracted in a Soxhlet apparatus successively for 10 h. with 200 mL of petroleum ether and ethanol, and concentrated in a rotary evaporator to obtain petroleum ether and ethanol extracts. The extracts were filtered and concentrated in a rotary evaporator. The concentrated extracts were dried in a desiccator containing calcium chloride. The dry extracts were kept in the dark below 4 °C in airtight containers.

Screening phytochemicals

Phytochemical screening was undertaken using standard methods [7].

Evaluation of antimicrobial properties

Preparation of nutrient broth and Muller-Hinton agar medium

Nutrient powder (1.3 g) was weighed and mixed with 100 mL of distilled water in a beaker. For Muller Hinton agar medium, Muller-Hinton powder (19 g) was weighed, mixed with 500 mL of distilled water. Each was stirred continuously to form a homogenous mixture medium.

They were further heated to aid the dissolution process, transferred into test tubes, plugged with cotton, and were sterilized in an autoclave at 15 psi at temperature of 121 °C for 15 min. After sterilization, the test tubes were kept in slanting position and incubated at 37.5 °C to preserve sterility.

Preparation of microbial sub-culture

Fresh microorganism culture was transferred into nutrient medium to create sub-culture microorganism. The microorganism culture was inoculated into sterilized nutrient medium using sterilized inoculation loop. The inoculant was incubated for 24 h at 37 °C in the incubation oven.

Determination of minimum inhibitory concentration (MIC)

The method of Balouiri *et al* was followed [8]. Two-fold serial dilution was performed on the extracts (20, 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16 mg/mL diluted DMSO). The extracts with equal amounts of nutrient broth were transferred into test tubes. Each tube was inoculated with a microbial inoculum prepared in the same nutrient medium. The tubes were incubated at 37 for 24 h °C. Minimum inhibitory concentration (MIC) was determined based on turbidity with 0.5 McFarland standard as reference.

Agar well diffusion assay

Agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. A hole with 8 mm of diameter was made using a sterile cork borer. A volume of 100 µL of extract of different concentrations (5, 10 and 20 mg/mL) was transferred using micro-pipette into the hole on the agar medium. The plate was incubated for 24 h at 37 °C. The diameter of the zone of inhibition was measured after the incubation period [8]. Tetracycline (50 µg) was used as positive control and dimethyl sulphoxide (DMSO) as negative control.

Anticancer screening

Plant extracts dilution

Ethanol extract of the fruit of *Synsepalum dulcificum* fruit (2 mg) was scraped out using a sterile spatula and weighed using an analytical mass balance before being put into a clean and sterile Eppendorf tube. The Eppendorf tube was then transferred into a laminar air flow and diluted with 1 mL DMSO to obtain a stock solution (2 mg/mL). The Eppendorf tube containing the extract was then sealed using aluminum foil. The stock solution was diluted with fresh media to obtain different concentrations (8.7, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) of the extract.

Preparation of cell culture media

The medium used in cell culture was RPMI (Roswell Park Memorial Institute) 1640 with 10 % FBS and 1 % penicillin-streptomycin as antibiotic source. RPMI (Roswell Park Memorial Institute) 1640 medium (250 mL) was prepared. FBS (10 %, 25 mL) was pipetted using a sterile serological pipette carefully. Penicillin - streptomycin (1%, 2.5 mL) was pipetted and added into medium using serological pipette. The Schott bottle was gently stirred to mix he supplements well with the medium. The volume of media was topped up to 250 mL. The prepared medium was stored at 4 °C in a refrigerator.

Cell culture

Colorectal cells (HCT-116 and PCE cell) were obtained from the Department of Medical Microbiology, University of Malaya (UM), Malaysia. The medium used was RPMI 1640 with 10 % FBS and 1% Penicillin-streptomycin. The cells were cultured in T25 flask at 37 °C in a humidified atmosphere at 37 °C in which the CO_2 level was maintained at 5% for 48 h.

Cell seeding

The confluency of cells was about 80 to 90 %. The medium was removed, and the cells were washed with PBS solution 3 times. The cells were then incubated with trypsin for 5 min to detach the cell from the surface and was centrifuged for 5 min. It was then removed from the tube and left with the pellet. The medium was added again into the falcon tube with pellets and mixed. Each exponential growth phase colorectal cancer cells was seeded, 1000 cells per well (HCT-116 and PCE), into 96-well plates and was incubated for 48 h. The number of viable cells per well was calculated using a haemocytometer.

Cell treatment

Different concentrations of extracts were prepared (8.7, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) were added into the 96-well plate. The plates were then incubated for 24, 48 and 72 h. Fluorouracil (10 µg/mL) was used as the positive control.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay

MTT (5 mg/mL) solution was added to the 96-well plate and incubated at 37 °C for 4 h. The cell suspension was then removed from each well carefully using micropipette. Dimethyl sulphoxide (100 µL) was added into each well and was covered with aluminum while shaking by using microtiter plate shaker for 15 min.

Quantitation of MTT cell proliferation assay

The 96-well plate was loaded into Glomax microplate reader to determine and record the absorbance of the content of each well using a wavelength of 570 nm with reference wavelength of 630 nm. The percentage of cell viability was determined based on the dose response relationship which describes the interaction and change in effect on the cells. The results are calculated as the percentage of viability in relation to the untreated cells.

Statistical analysis

All tests were carried out in duplicates. Data are expressed as mean ± SEM, and were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0. One-way analysis of variance (ANOVA) and post hoc test with Turkey HSD were conducted.

RESULTS

The percentage extract yields for petroleum ether and ethanol were 6.30 and 13.86 %. The results of phytochemical screening are summarized as in Table 1.

Table 2 shows the average zone of inhibition and standard deviation of different *Synsepalum dulcificum extracts* (petroleum ether and ethanol), tetracycline (0.05 mg/mL) and negative control (dimethyl sulfoxide) against different bacterial strains at different concentration.

Table 1: Phytochemical profile of extracts of the fruit of *Synsepalum dulcificum*

Variable	Test	Petroleum ether extract	Ethanol extract
Flavonoid	Alkaline	-	-
Alkaloid	Mayer's reagent	-	+
	Wagner's reagent	-	+
Phenolic	Ferric chloride	-	+
	Folin-Ciocalteau	-	+
Terpenoid	Salkowski	-	+
Tannin	Ferric chloride	-	+
	Diluted KMnO4	-	+
Glycoside	Modified Borntrager (C-glycoside)	+	+
Saponin	Foam	-	+
Fats and oils	Sudan Red III	-	-
	Benedict's reagent (reducing sugar)	+	-

- : not present; + : present in low concentration

Table 2: Zone of inhibition (mm) of different extracts of the fruit of *Synsepalum dulcificum* on different bacterial strains at different concentration

Extract (mg/mL)	Zone of inhibition (mm)					
	1	***2***	***3***	***4***	***5***	***6***
Petroleum ether						
5	7.98±0.59	7.52±0.15	7.33±0.01	7.55±0.06	9.35±0.19	8.00±0.92
10	8.86±0.63	7.63±0.07	8.20±0.14	6.92±0.22	9.97±0.51	7.89±0.27
20	9.28±0.91	7.76±0.05	8.35±0.09	7.93±0.19	8.79±0.49	7.13±0.31
Ethanol						
5	11.07±0.40	11.81±0.22	10.53±0.31	7.46±0.26	9.08±0.58	7.74±0.41
10	11.58±0.88	12.21±0.55	10.26±0.01	7.70±0.14	8.37±0.11	8.31±0.24
20	15.94±0.41	13.74±0.44	10.40±0.11	7.28±0.17	8.43±0.15	8.47±1.06
Positive control						
Tetracycline 0.05	44.12±0.50	36.11±0.55	38.49±0.43	8.75±0.55	41.17±1.00	34.52±1.02
Negative control						
DMSO	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Zone of inhibition = average ± standard deviation; 1 = *Staphylococcus aureus*; 2 = *Bacillus subtilis*; 3 = *Proteus vulgaris*; 4 = *Pseudomonas aeruginosa*; 5 = *Klebsiella pneumoniae*; 6 = *Escherichia coli*

Table 3: The minimum inhibitory values of extracts of *Synsepalum dulcificum* against selected bacteria strains

Bacterial strain	MIC of extract (mg/mL)	
	Petroleum ether	***Ethanol***
Staphylococcus aureus	0.156	2.500
Bacillus subtilis	0.156	0.313
Proteus vulgaris	0.156	0.625
Pseudomonas aeruginosa	1.250	0.156
Klebsiella pneumonia	0.156	0.625
Escherichia coli	0.156	0.313

The minimum inhibitory values for selected bacteria are shown in Table 3. Table 4 shows the results of the effect of the fruit of *Synsepalum dulcificum* on cell viability of HCT-116 cancer cells. The result of the effect of the fruit of *Synsepalum dulcificum* on cell viability of the primary colon epithelial (PCE) cells is shown in Table 5.

DISCUSSION

Results of the phytochemical screening showed that the ethanol extract of *Synsepalum dulcificum* contained more phytoconstituents than the petroleum ether fraction. A report of the quantitative phytochemical composition of *S. dulcificum* showed relatively high concentrations of flavonoids, tannins and saponins [9].

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentration (MIC) will inhibit growth but not necessarily kill the microorganism.

The MIC values of the two different extracts of *Synsepalum dulcificum* fruits against the growth of both gram-positive and gram-negative bacteria ranged from 0.156 to 2.500 mg/mL. For petroleum ether extract, all bacteria strains except *Pseudomonas aeruginosa* displayed the same high sensitivity. The growth inhibition effects of the extract on the bacteria was observed at 0.156 mg/mL. The petroleum ether

Table 4: Effect of the fruit of *Synsepalum dulcificum* on HCT-116 cell viability

Extract (µg/mL)	Viability of HCT-116 cell line (%)		
	24 h	***48 h***	***72 h***
Negative Control	100.00±0.00	100.00±0.00	100.00±0.00
Positive Control	59.63±0.69*	48.50±0.29*	29.95±0.95*
7.8	65.29±0.11*	52.02±0.12*	50.93±1.66*
15.63	48.65±0.62*	38.60±0.40*	41.14±0.51*
31.25	46.99±0.38*	34.52±0.97*	37.44±1.11*
62.5	45.64±0.53*	32.52±0.69*	35.14±0.56*
125	43.16±0.47*	29.48±0.44*	31.28±0.67
250	40.31±0.47*	25.84±0.47*	27.90±0.56
500	38.43±0.45*	23.40±0.33*	22.22±0.88*
1000	28.09±0.19*	18.84±1.47*	17.87±1.38*
Calculated IC_{50}	14.99 µg/mL	8.97 µg/mL	8.54 µg/mL

*Statistically significant difference at $p < 0.05$

Table 5: Effect of the fruit of *Synsepalum dulcificum* on the viability of primary colon epithelial Cells (PCE)

Extracts (µg/mL)	Viability of PCE cell line (%)		
	24 h	**48 h**	**72 h**
Negative Control	100.00±0.00	100.00±0.00	100.00±0.00
Positive Control	59.49±0.23*	47.83±0.51*	30.71±0.31*
7.8	83.21±1.34*	61.07±0.65*	71.52±1.81*
15.63	72.01±0.60*	58.12±0.93*	69.54±1.00*
31.25	70.48±0.77*	57.30±0.63*	68.04±1.58*
62.5	62.93±1.17*	54.31±0.97*	65.29±2.60*
125	55.99±1.02*	52.75±1.25*	61.51±0.96*
250	49.26±0.64*	48.52±0.61*	41.45±1.02*
500	45.11±0.05*	34.13±1.25*	37.33±0.77*
1000	41.01±1.00*	32.36±0.57*	32.09±2.46
Calculated IC_{50}	236.25 µg/mL	206.09 µg/mL	196.72 µg/mL

*Statistically significant difference at $p < 0.05$

extract had better microbial growth inhibitory activity than the ethanol extract. The results of zone inhibition of *Synsepalum dulcificum* fruits showed that the two extracts exhibited strong antimicrobial activities on all six bacterial strains. However, the zone inhibition produced by the ethanol extract was better than that of petroleum ether extract for *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris.* Essential oils obtained from the leaves of the plant have earlier been reported to exhibit antibacterial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus albus, Staphylococcus aureus, Micrococcus tetragenus, Micrococcus luteus, and Escherichia coli*. It is therefore possible that the observed antimicrobial effects of fruit of the plant may be attributed to essential oils present in the fruits.

Ethanol extract was used in the evaluation of anticancer activity on *Synsepalum dulcificum* fruits through MTT- assay against colorectal cell lines HCT- 116 and primary colon epithelial (PCE) cells because of its ability to dissolve polar and non - polar substances [10]. The results showed that ethanol fruit extract of *Synsepalum dulcificum* exhibited potent anticancer activities on both the cell lines after the treatment period (24, 48 and 72 h). In the test against HCT - 116 cell lines, the effect of the extract from the concentration of 15.63 to 1000 µg/mL was comparable with that of the positive control after 24, 48, and 72 h. While in the test against PCE cells, the result was comparable with that of the positive control at 24 and 48 h from 250 - 1000 µg/mL.

In had been reported that the methanol and ethanol extracts of stem, and ethanol extract of the berry of *S. dulcificum* were cytotoxic to HCT-116 and HT-29 human colon cancer cells, but none of the extracts was cytotoxic to the THP-1 monocytic leukemia cells and HDFn normal human dermal fibroblasts. For both HCT-116 and HT-29, these extracts were significantly up-regulated the expression of c-FOS and c-JUN, compared to the untreated negative control [11].

Phytochemicals such as polyphenols, flavonoids, alkaloids, and terpenoids are known to possess anticancer activity. The exact mechanism by which phytochemicals exert anticancer activity is still a topic of research. However, they are known to exert wide and complex range of actions on nuclear and cytosolic factors of a cancer cell [12,13].

Compounds that have been isolated from the fruit of the plant include 2-oxetanone, β-amyrin, α-amyrin, lupeol, oleanolic acid, and ursolic acid (6b, lupeol acetate, triglyceride, and linoleic acid [14].

The triterpenoids, ursolic and oleanolic acid, betulinic acid, celastrol, pristimerin, lupeol, and avicins possess antitumor property, while α-amyrin and β-amyrin possess antimicrobial activity [15,16].

The anticancer activity of the fruit of the plant may therefore be ascribed to the presence of lupeol, oleanolic acid, and ursolic acid in the fruit of the plant. Alpha-amyrin and β-amyrin may also contribute to its antimicrobial activity. However, further studies are required to confirm these.

CONCLUSION

The results indicate that petroleum ether extract of *Synsepalum dulcificum* fruits has superior antimicrobial activity to the ethanol extract. The ethanol extract also exhibits significant anticancer activity and may therefore be useful as an anticancer agent against colorectal cancer.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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