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Antimicrobial and Anticancer Activities *of Syzygium cumini* Extracts

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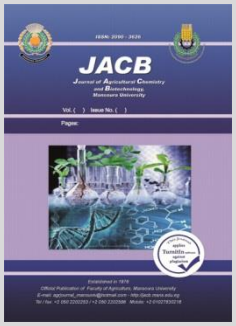

ABSTRACT

Syzygium cumini belongs to the family L. to Myrtaceae which are consumed as cherry-shaped fruits and are called pamposa in Egypt. The extracts of this fruit possess effective substances that have antimicrobial, antioxidant and anti-cancer effects. The methanolic extracts of S. cumini fruit and seeds were tested on three types of human cancer cells, and also as anti-microbial substances on six microorganisms, three bacteria and three fungal strains. The methanolic extract of unripe fruit pulp has the strongest inhibition (82.2%) against DPPH radical using 25 µg/mL while methanolic extract of ripe fruits pulp gave lowest inhibition value of 28.02% at the same concentration. The results showed that the methanolic extract (0.1%) of the pulp of the ripe fruits was more effective on microbial strains and fungal strains. The methanolic extract of unripe fruit seeds of *S. cumini* was showed that the best anticancer activity was obtained using the high concentration of the tested samples (1000 µg/ml) against colorectal adenocarcinoma (Caco2) with an IC50 value was 30.93.

***Keywords*: *Syzygium cumini*,** antimicrobial, colon, prostate, liver adenocarcinoma.

INTRODUCTION

Myrtaceae is a large family consisting of trees and shrubs found in tropical and subtropical regions and contains about 150 genera and 3600 species (Ayyanar *et al.*, 2012). *Syzygium cumini* L plant is found in Egypt on a limited scale distributed in the north and is also found in the Middle East and Southeast Asia (Chhikara *et al.*, 2018). The fruits and seeds of S. cumini have been used as a diabetes mellitus and anticancer treatment for centuries in South Asian folk medicine (Ruan *et al.*, 2008). On the other hand, some studies confirm that plant extracts from this plant have an anti-fungal and anti-bacterial effect on foods also bark is used in the treatment of diarrhea and dysentery (Kumar *et al.*, 2009). Moreover, *S.* cumini has sedative, antidepressant and potent central nervous system depressant effects (Srivastava and Chandra, 2013).

Plant extracts of S. cumini possess secondary active substances such as anthocyanins, phenols and tannins that have a high ability to kill microorganisms. (Ramya *et al.*, 2012).

Cancer is a public health problem worldwide. According to the World Health Organization, 20 million people in the world suffer from cancer, a number that is expected to rise to 30 million within 20 years. Therefore, solutions must be found away from chemotherapy and turning to natural materials and folk medicine (WHO, 2004).

Aspergillus sp. is one of the most common mycotoxins that have the ability to produce mycotoxins in food and feed. These mycotoxins are carcinogenic substances that settle or store in the livers of animals and humans, leading to liver damage (Jadhav *et al.*, 2009)

The aim of the current investigation was to evaluate the efficiency of *S. cumini* (L.) (pomposia) extracts and active ingredients as natural antioxidant, antimicrobial and anticancer activity.

MATERIALS AND METHODS

Collection and preparation of plant samples

Syzygium cumini fruits were collected from many areas in Damietta. The fruits were cleaned, washed with tap water, cut into fruit pulp and seeds, the samples were dried in the fridge for 7-10 days, and were dried in oven at 50°C for 3-4 days, then the dried samples were grinded and kept in polyethylene bags for analysis.

Preparation of methanolic extracts

Extraction was carried out according to a method according to Yadav *et al.*, (2017).

Chemical analysis of *Syzgium cumini* fruits pulp and seeds:

Moisture, Ash, protein and lipid content:

The chemical composition of pulp and seeds of *Szygium cumin* fruit was determined according to the method described by AOAC, (2007).

Phytochemical screening:

The crude methanolic extracts of *Syzgium cumini* samples were separately subjected to the following tests:

Detection of terpenes:

Terpenes were analyzed descriptively using Finar (1968).

Detection of tannins:

Tannins were analyzed descriptively using Gonzalez and Delgado (1962).

Detection of flavonoids:

Flavonoids were detected in the crude methanolic extracts according to the method of Geissman (1962).

Detection of resins:

The resins were analyzed descriptively using Harborne (1988).

Detection of saponins:

The saponins were quantified descriptively Trease (1961).

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Determination of total phenolic compounds

Total polyphenolic content of plant extracts was analyzed quantitatively using Folin-Ciocalteu reagent method according to Chang *et al.*, (2002).

Determination of total anthocyanins:

The anthocyanin content in the pulp and seeds of the fruit was quantified according to Mazumadar and Majumder (2003).

Ascorbic acid:

It was estimated into the dried seeds and pulp of cumin syzygium according to the method described in AOAC (2007).

Determined by using the die 2,6-dichlorophenol indophenols, method as described by Ranganna (1979).

Determination of reducing power:

The reducing power of the crude methanolic extract of *Syzgium cumini* samples was determined according to the method of Oyaizu (1986).

DPPH radical scavenging assay:

The measurement of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed according to method described by (Rekha *et al.*, 2012).

Antimicrobial assessment:

Microbial strains:

A spectrum of six microbial strains were as follow:

Bacteria: Three bacterial strains were used, including two strains Gram-negative namely *Escherichia coli* and *Salmonella typhi* and Gram-positive strains was *Bacillus subtilis*

Fungal strains: Three fungal strains that are pathogenic to plants and produce mycotoxins were used in this research: *Aspergillus oryzae*, *Penicillium notatum* and *Alternaria solani*.

Cultivation media

Cultivation was done on nutrient agar (NA) and potato dextrose agar (PDA) to grow bacterial and fungal strains, respectively. (El-Fadaly *et al*., 2018).

The agar diffusion method

This experiment used a method according to (El-Fadaly *et al*., 2018).

Determination of sample cytotoxicity on cells (MTT protocol)

Three types of cancer cells were used: HepG2, Caco2 and Pc3 cells method was carried out according to Slater *et al*., (1963)

RESULTS AND DISCUSSION

Chemical composition of investigated samples:

The moisture, crude protein, crude lipids and ash were determined in pulp fruit and seeds of green and fresh fruits of *syzygium cumini*. The results are recorded in Table (1).

Table 1. Chemical composition of investigated samples:

Components	Moisture %	Crude protein %	Crude Lipids %	Ash %	Total carbohydrate %
Ripe fruit pulp	85.01	2.94	0.51	0.4	11.14
Ripe fruit seeds	46.78	4.02	0.03	0.08	49.09
Unripe fruit pulp	79.26	4.34	0.42	0.1	15.88
Unripe fruit seeds	46.73	4.96	0.01	0.05	48.25

Phytochemical screening of *Syzgium cuimini* methanolic extracts:

Phytochemical screening are carried out on the crude methanolic extract of pulp fruit and seeds of green and fresh fruits of *Syzygium cumini*. From Table (2) it is clear that terpenes, tannins, flavonoids, phenolics glycosides and resins are detected in all the samples, however saponins are not found in all samples.

Table 2. phytochemical screening of crude methanolic extracts:

Samples	Terpenes	Tannins	Flavonoids	Saponins	Phenolics glycosides	Resins
Ripe fruit pulp	+++	++	+	+++	+++	-
Ripe fruit seeds	++	+++	+++	+	++	+++
Unripe fruit pulp	+	+	++	+++	+	+++
Unripe fruit seeds	++	+++	+++	+	++	+++

These results are consistent with Raza *et al.*, (2015) in ripe fruit pulp Where it was found that the ripe fruits contain Terpenes, Tannins, Flavonoids and Phenolics glycosides while the unripe fruits are rich resins.

Table 3. Total polyphenols, anthocyanin and ascorbic acid content of *Syzgium cuimini* methanolic extracts.

Samples	Total polyphenols	Total anthocyanins	Ascorbic acid mg/100g
Ripe fruit pulp	13.48	6.863	1350
Ripe fruit seeds	317.68	2.525	1500
Unripe fruit pulp	5.43	1.201	600
Unripe fruit seeds	217.68	1.608	700

From the previous Table3, the seeds of ripe fruits have a very high percentage of PShenols, and the ripe fruits have a high percentage of anthocyanins. These results apply with Gowri *et al.*, (2010).

Table 4. The inhibition percentage of the radical scavenger DPPH and IC_{50} values.

Samples	% Inhibition	IC_{50} Value
Ripe fruit pulp	28.02	57.7
Ripe fruit seeds	34.5	52.3
Unripe fruit pulp	82.2	21.10
Unripe fruit seeds	71.3	33.42
Vitamin C	90.2	10.3
Negative control	-	-

Results recorded DPPH had a maximum absorbance at 517 nM and an antioxidant concentration needed to reduce the initial DPPH concentration by 50% (IC_{50}). A lower IC50 value indicates a higher antioxidant power. The methanolic extract of the pulp of unripe fruit had the strongest inhibition (82.2%) against DPPH roots using 25 µg/ml while the methanolic extract of the pulp of the ripe fruit gave the inhibition value of 51.3% at the same concentration.

Table 5. Reducing power of *Syzgium cuimini* methanolic extracts.

Samples	% Inhibition	IC_{50} Value
Ripe fruit pulp	92.02	78.36
Ripe fruit seeds	41.00	329.62
Unripe fruit pulp	54.06	209.33
Unripe fruit seeds	42.30	305.3
Vitamin C	82.02	91.03

Data in Table 5 showed clearly that Ripe fruit pulp methanolic extract had the highest inhibition percentage 92.02% while, Un ripe fruit seeds gave the lowest inhibition value of 42.30%.

II. Antimicrobial effect of plant extracts

The plant extracts under study were tested against six microorganisms. This activity was assessed by the presence or absence of inhibition zones and the diameter in which no growth was observed was measured after incubation at 37 °C/48 h, 30 °C/72 h or 28 °C/7 days for bacteria and fungi, respectively. Results listed in Table (6) show the effect of crude methanolic extracts on the microbial strains either Gram positive or Gram negative bacterial and fungal strains. Results illustrated that methanolic extracts either in 0.05% or in 0.1% was more effective than the water extract on all the tested bacteria strains

Table 6. Values of areas of inhibition (mm) treated with methanolic extracts of *syzgium cuimini*

Bacterial strains	Methanolic extract (0.05%)				Methanolic extract (0.1%)			
	Ripe fruit pulp	Ripe fruit seeds	Unripe fruit pulp	Unripe fruit seeds	Ripe fruit pulp	Ripe fruit seeds	Unripe fruit pulp	Unripe fruit seeds
Bacillus subtilis	8.0	9.0	9.5	-	9.0	11.0	8.0	14
E. coli	10.5	11.0	-	8.0	25.0	20.1	11.0	8.0
Salmonella typhi	10.0	13	10.6	9.0	22.5	14.0	15.6	9.0

The results showed that the (G+)-forming *Bacillus subtilis* bacteria were more resistant than the (G-) bacterial strains when treated with the methanolic extracts. For (G-) bacterial strains, the results proved that *Salmonella typhi* was more resistant than *Escherichia coli* towards all tested extracts as shown in the same Table.

On the other hand, *Penicillium notatum* was more sensitive to most of the tested extracts as shown in Table (7).

Results recorded in Table (8) showed that the best anticancer activity was obtained using the high concentration of the tested samples (1000 µg/mL) against Caco2 colon, prostate and liver adenocarcinoma. Again, Unripe fruit seeds exhibited the highest activity. The IC_{50} value of Ripe fruit pulp methanolic extract was the lowest value 90.48 against Caco2 cell lines.

Table 7. Values of areas of inhibition (mm) treated with methanolic extracts of *syzgium cuimini*

Bacterial strains	Methanolic extract (0.05%)				Methanolic extract (0.1%)			
	Ripe fruit pulp	Ripe fruit seeds	Unripe fruit pulp	Unripe fruit seeds	Ripe fruit pulp	Ripe fruit seeds	Unripe fruit pulp	Unripe fruit seeds
Aspergillus niger	4.0	-	-	-	10	13.0	9.0	7.0
Penicillium notatum	6.0	5.0	-	-	9.0	12.0	8.5	7.5
Alternaria alternate	7.0	-	5.5	-	12.5	14.0	10.0	9.0

Table 8. % inhibition and IC50 values of methanolic extracts of *Syzgium cuimini* on three types of cancer cells.

Sample	Conc. µg/mL	Cancer cells Inhibition %			IC_{50}		
		HepG2	Caco2	Pc3	HepG2	Caco2	Pc3
Ripe fruit pulp	1000	96.56	97.38	97.16	24.78	90.48	51.21
	500	87.63	97.33	96.93			
	250	32.40	95.88	96.88			
Ripe fruit seeds	1000	96.62	97.22	96.82	89.1	39.48	38.3
	500	96.36	96.99	95.88			
	250	95.63	96.49	95.10			
Unripe fruit pulp	1000	96.69	97.32	97.16	77.33	40.21	53.71
	500	96.75	97.16	96.93			
	250	96.56	96.88	96.88			
Unripe fruit seeds	1000	96.69	96.71	95.99	75.2	30.93	43.21
	500	96.75	95.99	66.16			
	250	96.56	59.76	27.71			

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AOAC AOAC "Official Methods of Analysis, Association of Official Analytical chemists" (2007) 18th Ed

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الأنشطة المضادة للميكروبات والسرطان لمستخلصات نبات البامبوزا Syzygium cumini

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ينتمي Syzygium cumini إلى عائلة Myrtaceae حيث تستخدم ثمارها كفاكهة وتشبه فى شكل الكرز وتسمى البامبوزا في مصر, تحتوي مستخلصات هذه الفاكهة على مواد فعالة لها تأثيرات مضادة للميكروبات والأكسدة ومضادة للسرطان, تم اختبار المستخلصات الميثانولية لهذا النبات وبذوره على ثلاثة أنواع من الخلايا السرطانية البشرية ، وكذلك كمواد مضادة للميكروبات على ستة كائنات دقيقة وثلاث سلالات بكتيرية وثلاث سلالات فطرية. سلالة واحدة من البكتيريا موجبة لصبغة جرام (G +) ، العصوية الرقيقة ؛ سلالتين من البكتيريا سالبة لصبغة جرام (G-) ، السالمونيلا التيفية و *Eschrichia coli* ؛ كما تم اختبار ثلاث سلالات فطرية وهي *Aspergillus niger* و *Alternaria alternate* و *Penicillium natum* وقد ابرزت النتائج ان المستخلص الميثانولي للب الفاكهة غير الناضجة كان له أقوى تثبيط (82.2٪) ضد DPPH عند تركيز 25 ميكروغرام / مل بينما أعطى المستخلص الميثانولي للب الثمار الناضجة أقل قيمة تثبيط قدرها 28.02٪ بنفس التركيز و أظهرت النتائج أن المستخلص الميثانولي (0.1٪) من لب الثمار الناضجة كان أكثر فاعلية على السلالات الميكروبية سواء البكتيريا الموجبة لصبغة جرام أو السالبة لصبغة جرام وسلالات فطرية و أظهر المستخلص الميثانولي لبذور الفاكهة غير الناضجة أنه كان له أفضل نشاط ضد سرطان القولون تم الحصول عليه باستخدام التركيز العالي للعينات المختبرة (1000 ميكروغرام / مل) بقيمة IC_{50} كانت 30.93.

الكلمات الافتتاحية : البامبوزا ، مضادة للميكروبات ، مضادة للسرطان القولون ، سرطان البروستاتا ، سرطان الكبد