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Phytochemical profiling, antioxidant and anticancer activity of seeds extracts of *Syzygium cumini* (L.)

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Key words: *Syzygium cumini* (L.), Phytochemical profiling, DPPH, Antioxidant, Anticancer, Extraction

Abstract

Syzygium cumini (L.) is a common plant and known for various medicinal properties. The study was conducted to investigate the phytochemical profiling, antioxidant and anticancer activity from the seeds of *Syzygium cumini*. Ethanol was prepared from seed of *Syzygium cumini* used for phytochemical analysis. The qualitative phytochemical screening of the ethanolic extract revealed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, phenols and carbohydrates. The quantitative analysis proved the presence of total flavonoids, phenols analysed by standard methods. The present findings clearly demonstrated that *Syzygium cumini* was an effective antioxidant in various studies like vitamin C, vitamin E and *in vitro* antioxidant assays including DPPH, reducing rower assay and performed by standard protocols. The anticancer activity can be detected by using colon cancer (cell line HP 29). It can be done in ethanol extract of *Syzygium cumini* plant seeds. Therefore, the study indicates that the *Syzygium cumini* ethanolic extract has higher antioxidant and anticancer activity.

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Introduction

Syzygium cumini (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamun, etc. Different parts of the *jambolan* were also reported for its antioxidant, anti-inflammatory (8-11), neuropsychic-pharmacological, antimicrobial, anti-bacterial (De Castro and Priego-Capote, 2010), anti-HIV, antileishmanial and antifungal, nitricoxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexia-genic, gastroprotective and antiulcerogenic and radio-protective activities. A few of the current investigations have proved the selective cytotoxic activity of jamun fruit extract after studying its pro-apoptotic and antiproliferative effects on oestrogenin dependent (MDA-MB-231) breast cancer cells, oestrogen dependent/aromatase positive (MCF-7aro) and normal/ nontumorigenic (MCF-10A) breast cell line (Choi, 2008). Anti-cancer effects of 40% SC extract have been studied on human cervical cancer cells (11.8%growth inhibition observed in SiHa (HPV-16 positive) cells and 14.4% in HeLa (HPV-18positive) cells (Ramos *et al*., 2017) The leaf and seed extract of SC exhibited a significant antioxidant activity when they were assessed by various *invitro* methods such as Ferric reducing antioxidant power (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Nitric oxide radical scavenging, ABTS Assay, Total Reducing antioxidant potential, Total antioxidant activity, Reducing power and Hydroxy adical scavenging activity (Kabera *et al*.,2018).

The results obtained from the present study shows that the seed extract of *Syzygium cumini* possesses potent anti-oxidant, anti-diabetic and cytotoxic activities. The claim by traditional healers that the seed extract of *Syzygium cumini* is partially validated in the present study by identifying its ethanolic extract has high eralpha amylase and cytotoxic activity. If some of the compounds are structurally identified and characterized, they may be candidates for further antidiabetic and anticancer drug development.

Materials and methods

Plant material

All the chemical reagents used in this experiment were of analytical grade purchased from Loba chemicals, India. Fresh *Syzygium cumini* seeds were collected from Kancheepuram, Tamil Nadu. The seeds of *Syzygium cumini* were bought from local country drug store.

Preparation of ethanol extract

10g of *Syzygium cumini* dried seed powder was extracted with 200 millilitres of ethanol for 18 cycles using the Soxhlet apparatus. After 3hrs, the extract was concentrated in a rotary vacuum evaporator at temperature ranging from 30-40°C. The concentrates were dried in vacuum desiccators and the dried extract was stored at-20°C (Kabra and Patel, 2018).

Preliminary screening

Qualitative analysis of phytochemicals

A preliminary phytochemical screening of *Syzygium cumini* was carries out. The presence of alkaloids (Mayer'stest, Wagner's test and Dragendroff's test) (Senthilkumar *et al*., 2017), Flavonoids (Shinoda's test and alkaline reagent test) (Sharma, 2012), Tannins (Ferricchloride test and $K_2Cr_2O_7$ test) (Senthilkumar *et al*., 2017), Terpenoids (Salkowski test) (Senthilkumar *et al*., 2017), Polyphenols (Haroon *et al*., 2015), Proteins (Ninhydrin test) (Haroon *et al*., 2015), Saponins (Froath test, Lead acetate test) (Senthilkumar *et al*., 2017), Anthraquinone (Haroon *et al*., 2015), Glycosides (Dorntrage's test and Legal's test) (Senthilkumar *et al*., 2017), Carbohydrates (Senthilkumar *et al*., 2017), and Phytosterols (Libermann-Burchard's test) (Senthilkumar *et al*., 2017).

Quantitative analysis estimation of flavonoids

The standard Quercetin solution was added followed by the addition of 0.2ml of homogenate the solutions were made to equal volume using 3ml of 95% Ethanol. Then 0.1ml of Aluminium Chloride hexahydrate and 1M potassium acetate was added and incubated at room temperature for 40 minutes and the absorb anceisread at430 nm(Banerjee and Dasgupta, 2005).

Estimation of total phenol compounds

The amount of phenol was estimated by Folin-ciacalteau method (Brandão *et al.*, 2011).

Antioxidant analysis

Estimation of ascorbic acid (Vitamin C)

The level of ascorbic acid was estimated by the method of Omayeetal (Alikatte *et al.*, 2012).

Estimation of tocopherol (Vitamin E)

The level of tocopherol was estimated by the method of baker and frank (Ruan *et al.*, 2008).

Assay of catalase by inhale method

The activity of catalase (CAT) was determined by the method of Sinha (Bitencourt *et al.*, 2016).

Assay of superoxide dismutase activity

The assay of super oxided is mutase was done according to the procedure of Bitencourt *et al.* (2016).

Free radical scavenging activity

The antioxidant activity of the plant extract was assessed on the basis of the radical scavenging. Effect of stable 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) free radical activity by modified method. The scavenging reaction between DPPH andan antioxidant can be written as follows.

DPPH+H-A□DPPH-H+(A).

Yellow purple Antioxidants react with DPPH which is a stable free radical and is reduced to DPPH-H and as a consequence the absorbance deceased from DPPH radical to DPPH-H from. The degree of Discoloration indicates the scavenging potential of the antioxidant compounds of the extractsin Terms of hydrogen donating ability. The diluted working solution of the test extracts was prepared in methanol. In the test tube marked B, 2ml of methanol was taken. This was used to set zero. In the control tube, 2ml of DPPH was taken. In test, 1ml of the extract and 1ml of the DPPH were taken. These solution mixtures were kept in dark for 30 minutes and optical density was measure using 517nm using Spectrophotometer. The optical density was recorded and % inhibition was calculated using the Formula given below.

DPPH scavenging activity (%) = A control – A extract ×100

A control

Where control is the optical density of DPPH with methanol (El-Nashar *et al.*, 2021)

Determination of ferric reducing antioxidant power (FRAP)

The reducing power of plant extract was determined, according to the technique of Oyaizu (1986). Plant extract (10-100µg) in 1ml of distilled water was mixed and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 37°C for 20 minutes. 2.5ml of 10 % trichloroacetic acid was adding to the mixture, and the mixture was centrifuged at 1800rpm for 10 minutes. Then 2.5 ml of supernatant was mixed with 2.5ml of distilled water and 0.5ml of 0.1% $Fecl_3$. Absorbance was measured at 700nm, usinga UV-visible spectrophotometer (shimadzu, UV-2450). The reducing activity was calculated as % inhibition using the formula:

% Inhibition = ((A0-Ae) / Ao) ×100

Ao =Absorbance of without extract

Ae =Absorbance with extract / standard

Ascorbicacid was used as a standard antioxidant compound (Solai Prakash and Devaraj, 2019).

Inhibitory activity of alpha amylase

Assay of inhibitory activity of porcine pancreatic alpha amylase (Li *et al.*, 2021).

Treatment of cancer cell in with seed extract

The cell lines were treated with crude extracts, 1ml of MEM with Sodium pyruvate & 10% FCS was added to the dissolved *Syzygium cumini* aqueous extracts, Aqueous extracts were filtered through Whatman syringe filter (pore size-0.2µm). The filtered crude extracts weremade up of 1ml stock. 100µl of diluted *Syzygium cumini* aqueous extracts were added in the 96 well plates. The 96 well plates were kept in 5% CO_2 incubat or at 360°C and observed after 72hours (Banerjee and Narendhira kannan, 2011).

Cytotoxicity test of Syzygium cumini

The determination of cell cytotoxicity of *Syzygium cumini* leaf, seed and flower extracts on colon cancer cell lines (HCT15) was carried out, using a colorimetric assay that measures there duction of yellow -3-(4,5Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide) by mitochondrial succinate dehydrogenase enzyme described by Mosmann in 1983. The number of living cells and % of inhibition is directly proportional to the level of the mazan. The intensity of colour was quantified using a simple colorimetric assay. The results can be found out on a multi-well scanning spectrophotometer (ELISA reader) (Neha and Vibha, 2013).

FT-IR analysis

The *Syzygium cumini* were oven dried at 60°C and ground into fine ethanol extract using amortar and pestle. Two milligrams of the sample were mixed with 100mg KBr (FT-IR grade) and then compressed to prepare a salt disc (3mm diameter). The disc was immediately kept inthe sample holder and FT-IR spectra were recorded in the wavelength range between 500 and4000cm^{-1}.

GC-MS analysis

GC-MS is an ideal tool for identifying unknown or mixed substances that are present in very low quantities. The crude ethanol (1µl) extract of rhizomes containing different compounds of *Euphorbia hirta* was subjected for (GC-MS) analysis. The analysis carried out on a GCAGILENT system connected with mass spectrometer (MS). Helium used as a standard carrier gas. The sample is injected into an injection port of the GCMS. The column special oven which controls temperature range from 60°C to 300°C. Column heated to up to 300°C where the material is then volatilization. Inert capillary column size (30.0m×250µm).

Results and discussion

Phytochemistry is the branch of chemistry, deals with chemical properties of the plant or plant-derived products. It is also known as the chemistry of natural products. Phyto therapy acts as a source of treating and prevention of many diseases by using the bioactive compounds which are secondary metabolites of medicinal plants.

Phytochemical constituents of ethanolic seed extracts Syzygium cumini

Table 1 and Fig. 1 show the phytochemical composition of ethanol seed extract of *Syzygium cumini*. The extracts revealed the presence of Alkaloids, Flavonoids, Tannin, Saponins, Polyphenols, Terpenoids, Protein, Carbohydrates, and absence of anthraquinone, glycosides (Senthilkumar *et al.*, 2017) hephytochemical present in plant parts like leaves, stem, bark, roots, are used as medicines inserious disorders like heart-failure, cancer, blood pressure, etc., Euphoric and addicting drugs and pesticides or insect repellents (Nobori *et al.*, 1994).

Table 1. Preliminary phytochemical constituents of ethanolic seed extracts of *Syzygium cumini*

SL	Phytochemicals	Ethanol extract
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrate	+
4	Saponin	+
5	Tannin	+
6	Terpenoids	+
7	Proteins	+
8	Anthraquinone	-
9	Polyphenol	+
10	Glycosides	-

+Indicates present - Indicates absent

Fig. 1. Phytochemical constituents of ethanolic seed extracts of *Syzygium cumini*

Total phenolic and flavonoid content of ethanolic seed extract of Syzygium cumini

The total phenolic content is 5.8 (mg of gallic acid eq/g) and the total flavonoid contents is 1.8 (mg of quercetineq/g) (Table 2).

Previous studies have reported that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, since they act as antioxidants. This indicates that flavonoids which are subgroups of phenolic compounds had a lesser antioxidant activity than phenolic compounds which are the dominant contribute or to the antioxidant activity of the plant. Therefore, the Antioxidant activity of ethanolic seed extract *Syzygium cumini* contain sphenolic compounds (Banerjee and Dasgupta, 2005) other than flavonoids (Haroon *et al*., 2015; Ruan, 2008).

Table 2. Total phenolic and flavonoid content of ethanolic seed extract of *Syzygium cumini*

SL	Quantitative analysis of phytochemical	Ethanolic extract of seed
1	Phenol (mg/g of powdered sample)	5.8
2	Flavonoid (mg / g of powdered sample)	1.8

DPPH free radical scavenging activity

Table 3 shows free radical scavenging activity of ethanolic seed extract of *Syzygium cumini*. *Syzygium cumini* seed extract scavenges DPPH radical in the dose of dependent manner (250 µg to 1250µg). The percentage scavenging activity of *Syzygium cumini* seed increase with increase in concentration. However, the higher percentage (60.86%) scavenging activity was observed at1250 µg of concentration compared with standard ascorbic acid (El-Nashar *et al*., 2021).

Table 3. DPPH free radical scavenging activity

SL	Concentration (µg /ml)	% Inhibition of ascorbic acid	%Inhibition ethanolic extract of Seed
1	250	17.39	26.08
2	500	20.43	34.78
3	750	43.47	43.47
4	1000	56.52	52.17
5	1250	69.56	60.86

Ferrous reducing power antioxidant assay

Table 4 shows the reducing power assay (Solai Prakash and Devaraj, 2019). It is a convenient and rapid screening method for measuring the antioxidant potential. The reduction ability, i.e., "Fe^{3+} to Fe^{2+} transformation in terms of increase in absorbance at 700nm), was found to increase with increasing concentration of the extract. A maximum absorbance of 59% was obtained at a concentration of 500µg of extract. Gallic acid was used as positive control which gave maximum absorbance of 85% at a concentration of 500 µg.

Table 4. Ferrous reducing power antioxidant assay enzymatic antioxidant presents in the ethanolic seed extract of *Syzygium cumini*

SL	Concentration (mg/ml)	% Inhibition of ascorbic acid	%Inhibition ethanolic extract of seed
1	250	11	8
2	500	26	13
3	750	52	29
4	1000	65	47
5	1250	85	59

Table 5 shows the activity of catalase and superoxide dismutase. The catalase activity of ethanolic seed extract of *Syzygium cumini* is 2.7 mg of H_2O_2 utilized per minute. The super oxide dismutase activity of ethanolic seed extract of *Syzygium cumini* is 9.6mg of H_2O_2 utilized perminute (Bitencourt *et al*., 2016).

Table 5. Enzymatic antioxidant presence in the ethanolic seed extract of *Syzygium cumini*

SL	Enzymatic antioxidant activities	Crude homogenate of seed
1	Catalase (mg of hydrogen peroxide utilized/min)	2.7
2	Superoxide Dismutase (µg of Pyrogallol auto oxidation inhibition/min)	9.6

Non-enzymatic antioxidant presents in the ethanolic seed extract of Syzygium cumini

Table 6 shows the level of non-enzymatic antioxidant in the ethanolic extract of seed *Syzygium cumini*. The tocopherol present in the *Syzygium cumini* seed is 0.75mg/g of sample and ascorbic acid is 6.1mg/g. Phenolic compounds received considerable attention for their effective antioxidant properties, and their beneficial effects are attributed to their RP and FRSA affinity, Singh BN, Singh BR, Singh RL. Polyphenolics from ethanolic extract of seed *Syzygium* with potent antioxidant and antimutagenic activities. Plants are good sources of phenolic compounds.

Therefore, the plant which contains a high concentration of the antioxidant could be a good source of natural antioxidant. Numerous investigations of antioxidant activity of plant extracts confirmed a linear correlation between a total phenolic content and particulate antioxidant activity. That the phenolic compounds are generally better by using alcoholic solvents (Ruan *et al.*, 2008; Bitencourt *et al.*, 2016).

Table 6. Non-enzymatic antioxidant presents in the ethanolic seed extract of *Syzygium*

SL	Non enzymatic antioxidant activities	Crude homogenate of seed
1	Tocopherol (mg/g)	0.75
2	Ascorbic acid (mg/g)	6.1

FT-I R analysis of ethanolic seed extract of Syzygium cumini

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented. The peak at 2974.29 indicated alkenes (Table 7, Fig. 2). The peak at 2891.34 indicates C-H stretching of Alkane. The peak at 2365.73 indicates O-H stretching of carboxylic acid. The peak at 1969.35 indicates C=C=C Stretching of Allene. The peak at1649.17 indicates C= C Stretching, the peak at 1450.49, 1417.70, 1381.06, 1328.98, 1274.00, 1085.94 and 878.59 indicates, C-H Bending, S=O Stretching, S=O Stretching, S=O Stretching, C-O Stretching, C-O Stretching, and C-C Bending of, Alkane, sulphate, sulphonamide, sulfone, aromatic ester, aliphatic ether and alkene. FTIR absorption spectra of soluble extract reduction of Ag ions. Absorbance bands in the region of 500-4000 cm-1 are 3287, 2902, 1776, 1732, 1701, 1624, 1525, 1361, 1338, 1209, 1189 and 497cm-1. These absorbance bands are known to be associated with the stretching vibrations for O-H carboxylic acid, C-H alkane stretching, $(RC(O))_2O$ anhydride stretch, C=C alkenes stretch, C=O anhydride and ester stretch, $CH_3—C(=O)—CH_3$ ketone stretch, NO_2 nitro stretch, O-H stretching for alcohols and phenols, R–O–R ether stretch and mono-substituted benzene stretching. The total disappearance of this band the bio reduction since mainly responsible for the reduction of Ag ions, whereby they themselves to leading abroad peak at3287 cm^{-1}.

Table 7. FT-IR analysis of ethanolic seed extract of *Syzygium cumini*

Bond	Functional group	Frequency range
C-Hstretching	Alkanes	2974.29
C-Hstretching	Alkanes	2891.34
O-Hstretching	Carboxylicacid	2365.73
C=C=CStretching	Allene	1969.35
C=CStretching	Alkenes	1649.17
C-HBending	Alkane	1450.49
S=OStretching	Sulphate	1417.70
S=OStretching	Sulphonamide	1381.06
S=OStretching	Sulfone	1328.98
C-OStretching	AromaticEster	1274.00
C-OStretching	AliphaticEther	1085.94
C-C Bending	Alkene	878.59

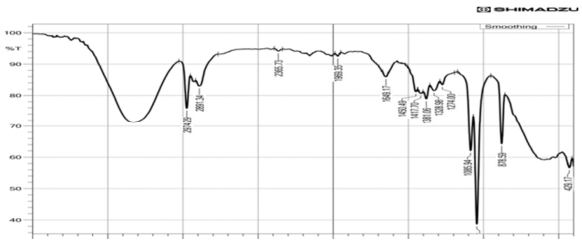

Fig. 2. FT-IR analysis of ethanolic seed extract of *Syzygium cumini*

GC-MS analysis of ethanolic seed extract of Syzygium cumini

The lead phyto components present in the ethanolic extract of *Syzygium cumini* seeds was identified by GC MS analysis. GC-MS chromatogram of ethanolic extracts *Syzygium cumini* there were 10 phytochemical compounds (1-Pentanol, Butanoic acid, 3methyl- Furfural, 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl, 5Hydroxymethyl furfural, Levomenol, trans-α-Bergamotene, Hexadecenoic acid, ethyl ester, n-Hexadecenoic acid, Hexadecenoicacid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester) were identified by using GC-MS analysis.

The different compound possesses following properties. Antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma, and diuretic and efficient antimicrobial agent (Table 8).

Table 8. GC-MS analysis of ethanolic seed extract of *Syzygium cumini*

SL.	Compound name	RT	Molecular weight	Molecular formula	Probability	Biological activity
1	1-Pentanol	4.342	88	C5H12O	70.2%	Anti-oxidant, corrosives inhibitor
2	Butanoicacid,3methyl-	4.437	102	C5H10O2	44.3 %	Anti-microbial, antioxidant
3	Furfural	4.972	96	C5H4O2	53.9 %	Anti-tyrosinase, anti-microbial
4	4H-Pyran-4-one,2,3dihydro 3,5dihydroxy-6-methyl	9.943	144	C6H8O4	92.1%	Anti-microbial, anti-inflammatory, Anti-oxidant hypoglycaemic
5	5Hydroxymethyl furfural	11.664	126	C6H6O3	89.6 %	Anti-proliferative, Anti-oxidant
6	Levomenol	16.416	222	C15H26O	4.89%	Anti-apoptotic, anti-inflammatory, anti-diabetic, Antioxidant
7	trans- α Bergamotene	18.679	204	C15H24	14.7 %	Anti-proliferative, anti-oxidant
8	Hexadecenoic acid, ethyl ester	19.491	284	C18H36O2	46.7 %	Hypoglycaemic, Hypocholesterolemic, Anti-oxidant
9	nHexadecenoic acid	19.565	256	C16H32O2	23.9 %	pesticides, nematicide
1 0	Hexadecenoic acid,2hydroxy-1(hydroxy methyl) ethyl ester	24.192	330	C19H38O4	41.6%	Haemolytic, flavour, pesticides, Anti-oxidant

Determination of alpha amylase inhibitory agents

The alpha amylase inhibitory studies performed demonstrated that the extracts of *S. cumini* seed had significant inhibitory potentials, which is about like that of voglibose drug.Voglibose is most effective and it is generally well tolerated in a wide range of type 2 diabetic mellituspatients (Table 9). These alpha amylase inhibitors are also are called as starch blocking as they prevent or slow down the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-Glycosidic linkage of starch and other oligosaccharide into maltose and other simple sugar (Li *et al.*, 2011). Cytotoxicity activity against colon cancer cell line (HT 29).

Table 9. Determination of alpha amylase inhibitory agents

SL	Concentration (µg/ml)	% Inhibition of voglibose drug	% Inhibition of seed
1	200	32.4	26.3
2	400	49.2	36.5
3	600	59.3	49.7
4	800	65.9	55.8
5	1000	81.2	63.9

Cytotoxicity activity against colon cancer cell line (HT29)

To evaluate the cytotoxic effect of the sequentially prepared extracts of *Syzygium cumini* seed extract on colon cancer cell line (HT29), the cells were treated with different concentrations of drug ranging from 12.5 to 100 µg/ml for 72 h of incubation and the cell viability was determined by MTT assay. Both aqueous and ethanol extract inhibited the viability in all thetested cell lines in a dose-dependent manner (Table 10).

Table 10. Cytotoxicity activity against colon cancer cell line (HT29)

Concentration (µg/mL)	Doxorubicin	ESC
50	26.81	17.95
100	65.17	28.16
150	78.86	39.75
200	86.38	48.43
250	96.23	55.59

Previous studies conducted evaluated the cytotoxic properties of *Syzygium cumini*. In their study, the seeds were separately extracted from *Syzygium cumini* in dichloro methane and the seed extracts were shown to induce a potent cytotoxic ability against different cancer cell lines, including HT-29. Inaddition, based on the MTT test, SCE suppresses the growth of HT-29 cell lines drastically. Expression analysis of the ratio of desired genes (Bax: Bcl-2) also changed significantly after treatment by SCE. It is interesting to observe the presence of several natural occurring cytotoxic compounds in *Syzygium cumini* and one of compounds, most elaborately studied is Gossypol. Further studies involving clinical trials showed minimal adverse effects, however the response rates of the receiving patients were low. Hence additional studies need to be performed to

explore either a new extractor a synergistic combination with an additional compound (Banerjee and Narendhira kannan, 2011).

Conclusion

The results obtained from the present study shows that the seed extract of *Syzygium cumini* possesses potent anti-oxidant, anti-diabetic and cytotoxic activities. The claim by traditional healers that the seed extract of *Syzygium cumini* is partially validated in the present study by identifying its ethanolic extract has higher alpha amylase and cytotoxic activity. If some of the compounds are structurally identified and characterized, they may be candidates for further antidiabetic and anticancer drug development.

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