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Original Article

CHEMICAL COMPOSITIONS, α-AMYLASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OILS FROM UNRIPE FRUIT PULP AND LEAVES OF *SYZYGIUM CUMINI*

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ABSTRACT

Objective: To investigate chemical compositions, α-amylase inhibitory and antioxidant capacity of the essential oils from the unripe fruit pulp and leaves of a traditional medicinal plant *Syzygium cumini*.

Methods: The essential oils of unripe fruit pulp and leaves of *S. cumini* were obtained by hydro-distillation and analyzed by GC-FID and GC-MS. *In vitro* α-amylase inhibitory and DPPH radical scavenging assay were carried out to study the antidiabetic and antioxidant activities of the essential oils.

Results: Thirty four components representing 99.3% of the unripe fruit pulp oil and 66 components representing 95.3% of the leaf essential oil were identified. α-cadinol (25.8%) and α-pinene (21.5%) were the major component of unripe fruit pulp and leaf oil respectively. The leaf oil showed better α-amylase inhibitory activity than unripe fruit pulp oil, while unripe fruit pulp oil exhibited higher antioxidant activity.

Conclusions: The mild α-amylase inhibitory and antioxidant activities of both oils are ideal for designing functional foods and can be used in food applications which aim to control diabetes.

Keywords: *Syzygium cumini*, Essential oil, α-Cadinol, α-Amylase inhibitory activity, Antioxidant activity.

INTRODUCTION

Diabetes Mellitus is a major metabolic disorder and about 90% of the diabetic patients in the world have been affected by Type 2 diabetes which is caused by abnormal carbohydrate metabolism and insulin resistance. Regulating production of glucose by reducing the activity of α-amylase and α-glucosidase, which are main involved in carbohydrate metabolism, is one of the mechanisms to control diabetes. The continuous use synthetic anti-diabetic drugs, which inhibit the activity of these enzymes, causes various side-effects [1-3]. Similarly, antioxidants play vital role in the body and protect from many health problems including diabetes by reducing oxidative damages. Again, the use of synthetic antioxidants has been reported to be unsafe to health [4, 5]. To avoid these harmful effects, efforts have been taken throughout the world to develop safe drugs from plants for various ailments including diabetes.

Essential oils mainly consist of mono and sesquiterpenes, which exhibit many biological activities. They have been reported to inhibit the activities of α-amylase and α-glucosidase [3]. Although, antioxidant activity of plant extracts is attributed to phenolics and flavonoids, many essential oils have been reported to possess antioxidant activity [6].

Syzygium cumini (L.) Skeels, (Myrtaceae), commonly known as jamun or Indian blackberry, is a medicinal plant used for various ailments in traditional medicines. The seeds and fruits are mainly used for diabetes [7]. However, leaves and bark have also been used for diabetes [8]. Anti-diabetic and antioxidant effects of the fruits and leaves of *S. cumini* have been attributed to the phenolics and flavonoids [9, 10]. But the fruits and leaves of *S. cumini* are also rich in essential oils. Mohamed *et al* [11], who studied the antioxidant activity of the crude extracts and essential oil of leaf of *S. cumini*, emphasised the need for further research on the essential oils of *S. cumini* to find out their use in food and pharmaceutical industries. Earlier studies reported the antibacterial, antioxidant and anti-inflammatory activities of leaf essential oils of this plant [11-15]. However, α-amylase inhibitory activity of both leaf and fruit essential oils and antioxidant activities of the fruit essential oil are yet to be investigated. Therefore, the present study was aimed to investigate the chemical compositions, *in vitro* α-amylase inhibitory and antioxidant activities of the essential oils from unripe fruit pulp and leaves of *S. cumini*.

MATERIALS AND METHODS

Plant material

Unripe fruits and leaves of *S. cumini* were collected from SASTRA University campus, Thanjavur, Tamil Nadu, India in the month of July. Herbarium voucher specimens (*R. Murugan 53*) were prepared for identification and deposited in the Herbarium of SASTRA University, India. The species was identified using regional Floras and confirmed by matching the specimens with the authentic herbarium specimens deposited in the Herbarium of the Royal Botanic Gardens (K), London.

Essential oil extraction

The seeds from the fresh unripe fruits were removed. Fresh unripe fruit pulp and leaves were subjected to hydro-distillation in a Clevenger apparatus for about 5 hours for extraction of essential oil. Colourless essential oils were obtained and dried over anhydrous sodium sulphate. The oils were stored under refrigeration at 4 °C and used for the analyses and assays.

GC-FID and GC-MS analyses

Gas Chromatography (GC) analysis was carried out using a PerkinElmer Clarus 500 Gas Chromatograph with Elite-5 capillary, non-polar column (30 m x 0.25 mm x 0.25 µm film thickness) coated with 5% phenyl and 95% dimethyl polysiloxane. The GC was fitted with Flame Ionization Detector (FID). The oven temperature was 60 °C - 240 °C at the rate of 3 °C/min. Injector and detector temperature was at 250 °C. Helium was used as carrier gas at a linear velocity of 30 cm/sec. and a pressure of 93.6 kPa. The flow rate was 1 ml/min. One µl of oil samples dissolved in hexane was injected. The split ratio used was 1:10. GC-MS analysis was carried out on a PerkinElmer Clarus 500 Gas Chromatograph using a non-polar, Elite-5 capillary column (30 m x 0.25 mm x 0.25 µm film thickness) coated with 5% phenyl - 95% dimethyl polysiloxane.

Oven temperature was initially 50 °C with 2 min. hold time and increased to 280 °C at the rate of 6 °C/min with a final hold time of 5 min. Injector temperature was 280 °C. Helium was used as the carrier gas at the rate 1 ml/min. The sample was dissolved in hexane and 1 µl of the sample was injected. The split ratio was 1:10. Mass spectra were recorded in the Electron Ionization mode at 70 eV in a scan range of 40-600 amu. Transfer line and ion source temperature were maintained at 200 °C and 150 °C respectively.

The compounds of the oil were identified by comparing the retention indices (RI) of the GC peaks obtained using homologous series of *n*-alkanes (carbon range from C_8-C_{20}) with those of compounds reported in literature. The mass spectra of the peaks were also matched with standards reported in literature [16] and the mass spectra of the compounds in NIST library. Peak area percentages were calculated from FID response without the use of correction factors.

α-Amylase inhibitory activity

In vitro α-amylase inhibitory assay was carried out according to Apostolidis *et al* [17] with slight modification. Starch solution (1% w/v) was prepared using 20 mM sodium phosphate buffer (pH 6.9). Various concentrations of essential oil solutions were prepared using DMSO. Initially, 100 µl of essential oil sample of various concentrations and 100 µl of α-amylase solution (0.5 mg/ml of 20 mM sodium phosphate buffer - pH 6.9) were incubated at 37 °C for 10 min. Then 100 µl of 1% starch solution was added to each tube and the mixtures were incubated at 37 °C for 10 min. The reaction was stopped by adding 200 µl of dinitrosalicylic acid colour reagent and tubes were incubated in a boiling water bath for 5 min. The mixtures were cooled to room temperature and diluted with 2 ml of distilled water. Each mixture was transferred to 96-well microplate and absorbance was measured at 540 nm. The experiments were performed in triplicates. The percentage inhibition was calculated using the below mentioned formula. The activity was also expressed in IC_{50} value as the concentration essential oil required to inhibit 50% of α-amylase.

$$\% \text{ inhibition} = [A_c - A_s / A_c] \times 100$$

Where, A_c is absorbance of control and A_s is absorbance of the sample.

Antioxidant activity

Antioxidant activity of the essential oils of *S. cumini* were determined using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay [17]. Different concentrations of essential oil solutions were prepared using methanol. The assay mixture contained 100 µl of essential oil samples, and 900 µl of methanolic DPPH (100 µM). The mixture was incubated for 30 min. at 25 °C in dark condition. Absorbance was measured at 517 nm. Antioxidant activity of the essential oils was calculated as follows. IC_{50} values were calculated as the percentage of essential oils required to scavenge 50% of DPPH free radicals.

$$\% \text{ inhibition} = [A_c - A_s / A_c] \times 100$$

Where, A_c is absorbance of control and A_s is absorbance of the sample.

Statistical analysis

The data were analyzed by using one way analysis of variance (ANOVA). The SPSS for Windows (version 16.0) was used for statistical analysis. The results were given as mean ± standard deviation.

RESULTS

Chemical compositions of the essential oils

Essential oils of the unripe fruit pulp and leaves of *S. cumuni* were analyzed by GC-FID and GC-MS. Thirty four components constituting 99.3% of the oil of unripe fruit pulp and sixty six components constituting 95.3% of the leaf essential oil were identified (Table-1). Both the oils were found to be rich in monoterpenes. The essential oil of unripe fruit pulp contained monoterpenes (41.9%), oxygenated monoterpenes (10.7%), sesquiterpenes (18.2%) and oxygenated sesquiterpenes (28.1%). The leaf oil contained monoterpenes (38.8%), oxygenated monoterpenes (21.5%), sesquiterpenes (26.0%) and oxygenated sesquiterpenes (8.6%). The major components of the unripe fruit pulp oil were α-pinene (12.4%), β-pinene (8.0%), myrcene (8.4%), α-terpinessential oill (7.4%), δ-cadinene (7.7%) and α-cadinol (25.8%). The principal components of leaf essential oil were α-pinene (21.5%), *trans*-ocimene (6.8%), α-terpinessential oill (9.5%) and δ-cadinene (8.3%). The compositions of these two oils showed much difference although a few components were present in both the oils. Many minor components identified in the leaf essential oil were not detected in the oil of unripe fruit pulp.

Table 1: Composition of the essential oil of unripe fruit pulp and leaves of *Syzygium cumini*

S. No.	Compound	RI	Area %	
			Unripe Fruit Pulp	Leaf
1	*cis*-3-Hexenol	856	tr	0.3
2	α-Thujene	929	-	1.0
3	α-Pinene	945	12.4	21.5
4	Camphene	952	0.3	0.4
5	Sabinene	974	-	0.1
6	β-Pinene	978	8.0	0.1
7	Myrcene	992	8.4	2.7
8	α-Phellandrene	1002	1.2	0.5
9	δ-3-Carene	1005	-	0.6
10	Limonene	1029	8.9	1.7
11	β-Phellandrene	1033	-	0.3
12	*cis*-Ocimene	1037	0.6	1.5
13	*trans*-Ocimene	1048	-	6.8
14	γ-Terpinene	1057	0.6	0.5
15	Terpinolene	1087	1.4	0.5
16	6-Camphenone	1095	-	0.2
17	Linalool	1097	0.6	2.0
18	*endo*-Fenchol	1115	-	0.4
19	*allo*-Ocimene	1118	0.1	-
20	1-Terpinessential oill	1124	0.5	-
21	*cis*-Epoxyocimene	1133	-	0.3
22	*trans*-Pinocarvessential oill	1142	-	tr
23	Camphene hydrate	1157	0.2	0.4
24	*cis*-Pinocarvessential oill	1159	-	0.4
25	Bornessential oill	1174	tr	0.4
26	Terpinen-4-ol	1178	1.8	2.7
27	Myrtenal	1189	-	0.2
28	α-Terpinessential oill	1196	7.4	9.5
29	*endo*-Fenchyl acetate	1204	-	1.0
30	*trans*-Carvessential oill	1215	tr	-
31	*trans*-Linalool oxide acetate (Pyranoid)	1284	-	0.2
32	Bornyl acetate	1291	-	2.0
33	*trans*-Pinocarvyl acetate	1298	-	1.1
34	*cis*-Pinocarvyl acetate	1303	-	0.3
35	α- Cubebene	1347	-	0.1
36	*trans*-Myrtenyl acetate	1350	-	0.1
37	Neryl acetate	1359	0.2	0.2
38	Cycloisosativene	1368	-	0.1
39	α-Copaene	1376	-	0.7
40	β-Cubebene	1383	0.3	-
41	β-Elemene	1386	0.2	0.2
42	α-Gurjunene	1406	0.2	-
43	Caryophyllene	1416	1.5	1.6
44	α-Humulene	1453	1.8	2.5
45	*allo*-Aromadendrene	1462	1.2	0.8
46	γ-Gurjunene	1470	-	0.5
47	γ-Muurolene	1475	-	0.9
48	Germacrene D	1481	1.8	0.9
49	Valencene	1491	-	0.4
50	α-Muurolene	1498	2.7	2.8
51	β-Dehydroagarofuran	1507	-	0.5
52	γ-Cadinene	1515	-	2.1

53	δ-Cadinene	1523	7.7	8.3
54	*cis*-Calamenene	1525	-	1.0
55	Cadina-1, 4-diene	1533	0.8	2.1
56	α-Cadinene	1537	-	0.9
57	α-Calacorene	1542	-	0.2
58	Germacrene-D-4-ol	1573	0.8	-
59	Caryophyllene alcohol	1577	-	0.2
60	Caryophyllene oxide	1581	-	0.4
61	Ledol	1601	-	1.2
62	Humulene epoxide II	1604	-	2.1
63	1, 10-*Diepi*-Cubenol	1610	1.2	0.4
64	γ-Eudesmol	1620	-	0.6
65	1-*epi*-Cubenol	1622	0.3	1.2
66	Cubenol	1639	-	0.2
67	τ-Cadinol	1642	-	0.2
68	Selina-3, 11-dien-6α-ol	1651	-	0.1
69	α-Cadinol	1658	25.8	1.6
70	Selina-6-en-4α-ol	1662	-	0.2
71	α-Bisabolol	1674	-	0.1
72	Selina-7(11)-en-4β-ol (Juniper Camphor)	1700	-	0.3
73	Benzyl benzoate	1780	0.4	-
Total			99.3	95.3

RI – Retention Index; tr - < 0.1%

Though major compounds such as α-pinene, β-pinene, α-terpinessential oill and δ-cadinene were present in both the oils, myrcene and α-cadinol identified as major compounds in the unripe fruit pulp were present as minor compound in leaf oil. However, *trans*-ocimene, a major component of leaf essential oil, was not detected in unripe fruit pulp oil.

α-Amylase inhibitory activity

The essential oils of unripe fruit pulp and leaves of *S. cumini* exhibited concentration dependent α-amylase inhibitory activity. The essential oils exhibited mild α-amylase inhibitory activity. The leaf essential oil showed better inhibitory effect than the unripe fruit pulp oil (Table 2). The IC_{50} values of both the leaf and unripe fruit pulp essential oils were found to be more than 1000 μg/ml.

Antioxidant activity

The results of DPPH radical scavenging effects of the essential oils of unripe fruit pulp and leaves of *S. cumini* are shown in table 2. The amount of unripe fruit pulp and leaf essential oils needed for 50% inhibition of free radicals (IC_{50} values) was found to be 219 and 357 μg/ml respectively. The unripe fruit pulp oil exhibited slightly better activity than the leaf oil. However, at higher concentrations (1000 μg/ml), the degree of activity was found to be equal in both the oils.

Table 2: α-Amylase inhibitory and antioxidant activity of unripe fruit pulp and leaf essential oils of *Syzygium cumini*

Concentration of the oil (μg/ml)	α-Amylase inhibition* (%)		Antioxidant activities** (%)	
	Unripe Fruit Pulp oil	Leaf oil	Unripe Fruit Pulp oil	Leaf oil
62.5	18.0±6.24	33.6±4.49	56.4±2.39	52.5±0.61
125	20.5±3.97	38.9±3.24	59.2±2.30	54.8±1.07
250	21.8±4.86	40.9±3.16	61.7±2.86	57.0±1.14
500	28.1±6.11	44.9±3.52	62.4±1.49	59.6±1.02
1000	37.2±7.84	52.4±6.91	63.8±1.22	62.9±1.00

Results were mean ± SD of triplicate (*n = 4; ** n = 3).

DISCUSSION

This is first report on the chemical composition of the essential oil of unripe fruit of *S. cumini*. However, the compositions of the essential oils of ripe fruit have been reported and contained α-pinene (30.8%), β-pinene (10.8%), *cis*-ocimene (18.5%) and *trans*-ocimene (12.1%) [18]; myrcene (6.9%), *cis*-ocimene (29.9%), *trans*-ocimene (23%) and α-terpineol (6.4%) [19] as the major components. The chemical compositions of the essential oils of ripe and unripe fruits of *S. cumini* widely differed. The major components α-cadinol and δ-cadinene present in unripe fruit pulp essential oil, were not reported in the essential oils of ripe fruits. Also, *trans*-ocimene reported in the ripe fruit essential oil of earlier studies was not present in the unripe fruit pulp oil. Similarly, the composition of leaf essential oil in the present study differed considerably from the earlier studies. α- and β-pinene, *cis*-, *trans*- and *allo*-ocimene, α-terpineol, 1, 3, 6-octatriene, caryophyllene, α-humulene, *epi*-globulol and caryophyllene oxide have been reported as major components in earlier studies from different regions [11-13, 15, 18, 20]. δ-cadinene reported in the present study was not reported earlier.

Anti-diabetic activities of the fruits and leaves of *S. cumini* have been least investigated, though the seeds and kernels have been considerably studied. Earlier studies showed that the water extract of ripe fruit pulp showed significant hypoglycemic activity than ethanol extract [21, 22]. However, other studies on the ripe fruits showed that ethanol extract significantly activity [23, 24]. The leaf decoction and extracts of *S. cumini* were found to have no anti-diabetic activity on rats and patients [25-27]. However, the ethanolic crude extract and aqueous extract of the leaf showed significant hypoglycemic activity in rats [28, 29]. In the present study, leaf essential oil demonstrated mild α-amylase inhibitory activity. The excessive inhibition of α-amylase activity is believed to cause flatulence and diarrhea due to irregular bacterial fermentation of undigested carbohydrates in the colon. Therefore, mild inhibition of α-amylase has advantage over complete inhibition [2]. Therefore, the essential oils of unripe fruit pulp and leaf of *S. cumini* may be ideal for developing functional food focusing on anti-diabetic potential.

Earlier studies showed that ripe fruit skin, aqueous extract of ripe fruit pulp, ethanol, ethyl acetate and acetone extracts of fruit and leaf found to have significant antioxidant activity [22, 30-33]. Mohamed *et al* [11] have reported methanol extract of the leaf to have high antioxidant activity than the leaf essential oil. In another study, leaf essential oil showed highest scavenging activity than solvent extracts [14]. Generally, antioxidant activity of plants has been ascribed to phenolics and flavonoids [9]. Srivastava and Chandra [10] have attributed the antioxidant effects of the leaf of *S. cumini* to the phenolics and flavonoids. However, Reddy and Jose [14] reported higher antioxidant activity in the leaf essential oil than the phenolics and flavonoids rich solvent extracts of the leaf of *S. cumini*. Elansary *et al* [12] also reported that the leaf essential oils exhibited significant antioxidant activity. In the present study, leaf essential oil showed good antioxidant activity. It is interesting to note that various extracts of the leaf of *S. cumini* exhibited antioxidant activity. This could be due to synergic effect of many compounds present in the leaf of *S. cumini*.

CONCLUSION

There is a high demand for natural compounds for treating various ailments. The present study was an effort to evaluate the potential of the essential oils of unripe fruit pulp and leaf of *S. cumini* for α-amylase inhibitory and antioxidant activities. The study demonstrates that both essential oils are capable of inhibiting α-amylase activity, however leaf oil has better inhibitory activity than the unripe fruit pulp oil. The mild α-amylase inhibitory and antioxidant activities of both oils are ideal for designing functional foods and can be used in food applications which aim to control

diabetes. However, further *in vivo* research is needed to assess and ascertain these activities of the essential oils of the plant.

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CONFLICT OF INTEREST

We declare no conflict of interest

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