Original Research Article

Antioxidant property of Palmyra palm *(Borassusflabellifer)* crunchy kernel– A potential Nutraceutical Food

ABSTRACT

Palmyra palm (*Borassusflabellifer)* commonly used all over the world for culinary purposes is said to have a lot of nutraceutical properties like antioxidant, anticancer activities, etc. Free radical formation in the cells of living organisms has been linked to cancer, atherosclerosis, and vision loss. An intake of antioxidants is believed to reduce these risks and helps in cell rejuvenation activities. The antioxidant capacity of methanolic extract of palmyra palm fruit crunchy kernel was estimated through DPPH method. The GC/MS analysis of crunchy kernel solvent extracts *viz.*, methanol and hexane exhibited various nutraceutical compounds like 5 - Hydroxy methyl furfural, Beta - D- Glucopyranose 1,6-anhydride, Linoleic acid ethyl ester, 9- octadeceneic acid ethyl ester, Butylated hydroxytoluene and Stigmasterol. Based on the results, the crunchy kernel is found to have possesses immense antioxidant potential and due to which it could be exploited effectively as value added food product.

Comment [uM1]: Author(s) used different parts of palyra palm to evaluate their antioxidative properties using the DPPH assay, not just the fruit crunchy kernel.

Keywords: *Borassusflabellifer*; DPPH method; GC/MS analysis; antioxidant

1. INTRODUCTION

Plants provide the basic diet for over 5 billion people in the world(1). Western civilization relies on about six major staples (Wheat, potato, rice, oats, barley and corn) as their source of dietary energy. This reduction in plant use has both nutritional and agricultural implications. A reduction in use of plant biodiversity means a significant loss of valuable food sources to mankind and leads to deficiency in nutrient requirement of mankind.

Many fruits, vegetables and tubers have been consumed by humans since ancient times. Scientific investigations have proved that an increased consumption of these, have several health promoting as well as disease preventing benefits because of certain substances known as phytochemicals which include polyphenols, vitamins, minerals, proteins, etc. The presence of polyphenols in fruits, vegetables and tubers is largely influenced by genetic factors and environmental conditions. The dietary phytophenolics like flavonoids and phenolic acids have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species, including O^{2-}, H_2O_2, -OH and O_2 (2).

Dietary recommendations for the prevention of cancer, atherosclerosis and other chronic diseases have been established by various health agencies. In living organisms, oxidation is essential for the production of energy to fuel biological processes. However, oxygen-cantered free radicals and other

reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes, cardiovascular disease and aging (3).

Because oxidation is a naturally occurring process within the body, a balance with antioxidants must exist to maintain good health. Antioxidants that scavenge these reactive oxygen species and free radicals are of major importance in preventing the onset and progression of many diseases caused by oxidative stress. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very effective and are used for industrial processing, but they may possess side effects and toxic properties that affect human health 1983 (4, 5, 6).

The search for antioxidants from natural sources has received much attention and efforts have been put into the identification of compounds that can act as suitable antioxidants and quantify these important compounds in natural sources so as to evaluate their nutraceutical potential and health benefits. Thus, investigation and characterization of various nutrients, phytochemicals and other activities present in various parts of *Borassusflabellifer* is the aim of the present study in order to understand their health benefits. Analysing the composition and bioactive compounds present in the crunchy kernel can lead to a better understanding and appreciation of the nutraceutical and medicinal value, this might offer an increased consumption by the general public.

Borassusflabellifer is a tall tree attaining a height of about 30 m, with a black stem and crown of leaves at the top; leaves are 0.9-1.5 m in diameter, palmately fan shaped, petiole edges with hard horny spinescent serrates; flowers are unisexual; fruits are large. Trees can live more than 100 years. The rate of growth has been estimated at about 3cm per year. This plant is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc. (7, 8,9).

The seedlings as well as the fleshy roots are eaten. These acts as an important item of diet for the poor. About 100 to 150 drupes are sown in 3-4 layers per 0.8 sq. m under loose and sandy soils, which may produce at least 100-150 seedlings, sometimes more. They are removed when 2-4 months old and the elongated, club-shaped, starchy, tender material is eaten either baked, roasted, fried or boiled, or made into flour. The fleshy roots are eaten as a fibrous and nutritious food when about four months old. They are rich in starch, but poor in fats and proteins. The germinated seed's hard shell is also cut open to take out the crunchy kernel which tastes like water chestnut but sweeter. Studies on this plant have revealed the presence of several steroidal saponins (7,8,9), a polysaccharide (10), and a triterpene (11). The fresh pulp is reportedly rich in vitamins A and C (12) while the fresh sap is a good source of vitamin B-complex (13). No information is available regarding antioxidant activity of the crunchy kernel. Therefore, the present study was carried out to evaluate nutritional quality, particularly with respect to antioxidant property of crunchy kernel.

Comment [uM2]: The results of the research show only the antioxidative properties by DPPH assay and the minor chemical compounds in the crunchy kernels, but not the major nutrients or nutritional compositions.

These biochemicals are often referred to as Secondary metabolites which is useful to traditional medicine system and these biochemicals are identify by using GC-MS technique. In recent years Gas chromatography – Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of essential oil,alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds (14). Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer (15) and Alzheimer's diseases (16).

2. MATERIALS AND METHODS

2.1 Plant material collection

The experiment was carried out at AC and RI, Madurai. The different parts of *B. flabellifer* such as Root, Root cover, Crunchy kernel, Leaf, Calyx, Pericarp and fruit were collected from the local, Madurai district, Tamil Nadu. The collected materials were washed with distilled water and then dried under shade. The materials were further dried in hot air oven at 55^0C to reduce the moisture content. The dried materials were subjected to size reduction to a coarse powder and stored at room temperature (28±2°) in air tight plastic bags for solvent extraction.

Comment [µM3]: Author(s) should provide the amount of moisture content of the dried materials.

2.2 Preparation of plant extract

The powdered samples were continuously extracted with solvents such as hexane and methanol separately by sonicating the samples along with solvents for 30 minutes.Then the samples were centrifuged at 10,000 rpm and filtered. The volume of the samples was concentrated by using rotary evaporator at 35-40ºC under reduced pressure.

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2.3 DPPH radical scavenging property

The 2,2-diphenylpicryl-1-picryl-hydrazyl radical (DPPH) scavenging activity of Palmyrah crunchy kernel extracts was measured according to the method of Blios (17).

Comment [µM5]: Author(s) should provide sample preparation and the amount of extract used for evaluation of DPPH and GC-MS analysis.

2.4 GC-MS analysis

The GC-MS analysis was carried out using Shimadzu QP 2020 instrument with RxSil 5MS column (0.25 mm × 30 m × 0.25 µm).The oven temperature was raised from 80°C upto 280°C, Injection port temperature was ensured as 280°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 1:10. Mass Spectral scan range was set at 50-500 (m/z). Transfer line and source temperature: 260ºC, 270ºC, Library: NIST 2005, Sample injected: 1.0µL

The two extracts namely hexane and methanol were diluted with their respective solvents (100 mg ml^{-1}) and analysed by GCMS. The chromatographic separation was done on a capillary column of fused silica RxSil-5ms (0.25 mm × 30 m × 0.25 µm). 1 µl of each extract was injected in the split mode (1:10). Eluents were detected in EI mode with ionization energy of 70 eV. All the mass spectra of the

identified peaks were compared with the spectra from the NIST 2014, WILEY spectral library and F.A.M.E mix (C8:C24) in combination with deconvolution reporting software (DRS). The results for individual compound those quality matches > 90% is only reported (as their percentage of the total area of peaks in the total ion chromatogram) (18).

3. RESULTS AND DISCUSSION

3.1 DPPH radical scavenging property

The results on DPPH radical scavenging activity of the different parts of Palmyra palm in methanolic extract along with the reference standards butylated hydroxyl toluene (BHT) and ascorbic acid are shown in table. 1. The model of stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. As radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH molecule, the colour change from purple to yellow.This leads to decrease in absorbance. Therefore, lower value indicates a higher antioxidant activity.The formula for calculating the antioxidant activity is as follows

%AAT = [(controlabsorbance. - Sample absorbance.)/Control absorbance.]* 100

Table 1. Radical scavenging capacity of Crunchy kernel of *Borasusflabellifer*L.

S.No.	Sample	Absorbance	Antioxidant activity	DMRT Ranking
1	Control	0.7636	0	
2	Root	0.6123	19.81404	c
3	Root cover	0.3865	49.38449	b
4	Crunchy kernel	0.6740	11.73389	c
5	Leaf	0.1128	85.22787	a
6	Calyx	0.0623	91.84128	a
7	Pericarp	0.0733	81.03493	a
8	Fruit	0.5055	33.80042	b

Methanolic extract of crunchy kernel showed higher levels of free radical scavenging activity among the solvent extracts tested. The DPPH radical scavenging activity was found to be the least in calyx and leaves. The scavenging effect of extracts of various parts with the DPPH radical is in the following order: Crunchy kernel > Root > Fruit pulp > Root cover > Pericarp > Leaf > Calyx.

3.2 GC-MS analysis

Plants are a tremendous source for the discovery of new products of drug development. Today several distinct chemicals derived from plants are important drugs that are currently used in more countries in the world (19). Medicinal plantsserves as potential source of therapeutic aids and also plays

Comment [uM6]: Capital letter.

Comment [uM7]: This sentence and formula should be in Materials and Methods section.

Comment [uM8]: According to DMRT ranking, DPPH radicals between crunchy kernel and root are not significantly different. Why did author(s) select only crunchy kernel for further experiment of GM-MS analysis.

Comment [uM9]: Author(s) should discuss DPPH assay results for the crunchy kernel and compare them with findings from other studies on related parts of the palmyra palm. This comparison would provide a broader context for the results and enhance the manuscript's overall depth.

a significant role in health system of both humans and animals. Plant based drugs remain an important source of therapeutic agents because of their relatively cheaper cost, availability and non-toxic nature when compared to modern medicines (20). The preliminary antioxidant screening tests (DPPH method) may be useful in the detection of presence of bioactive principles (Table 1.) GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc (21). The present study carried out GC-MS chromatogram of the hexane and methanolic extract of Crunchy kernel of *Borassusflabellifer* showed 50 major peaks respectively. (Table 2a and 2b) The presence of various phytocomponents have been identified after comparison of the mass spectra with NIST library.

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Comment [µM11]: Delete '-'.

Table 2a. List of compounds in Hexane fraction of Crunchy kernel detected by GC/MS

S.No	Peak Name	Retention Time(min)	Area	Area%
1	Cyclohexane,1,3-dimethyl-,trans-	3.143	150335	0.50
2	Oxirane,2,2-dimethyl-3-propyl-	3.771	58648	0.19
3	1-Propene,2-methyl-3-propoxy-	3.929	70405	0.23
4	Phosphonousdibromide,cyclohexyl-	4.305	332725	1.10
5	D-Limonene	4.886	173653	0.58
6	Undecane,5,7-dimethyl-	5.025	64283	0.21
7	Benzene,1,3-bis(1,1-dimethylethyl)-	6.815	225152	0.75
8	Dodecane,2,6,11-trimethyl-	7.006	157050	0.52
9	Dodecane,4,6-dimethyl-	7.515	93562	0.31
10	Hexadecane	8.506	150154	0.50
11	D-Alanine,N-neopentyloxycarbonyl-,octadec	9.393	194669	0.64
12	2,5-cyclohexadien-1-one,2,6-bis(1,1-dimethyl	9.488	363067	1.20
13	2-Tridecenal,(E)-	9.634	132559	0.44
14	Dodecane,4,6-dimethyl-	9.835	66391	0.22
15	Heptadecane	9.883	316030	1.05
16	Pentadecane	10.047	378891	1.25
17	Acetone	10.110	115493	0.38
18	ButylatedHydroxytoluene	10.255	3403009	11.27
19	Hexadecane	10.659	130710	0.43
20	Dodecanoic acid	11.033	139911	0.46
21	Hexadecane	11.887	462736	1.53
22	Heneicosane	14.000	569822	1.89
23	Phosphorin,2,4,6-tris(1,1-dimethylethyl)-	14.639	167283	0.55
24	Eicosane	14.965	226832	0.75
25	Tetradecanoicacid	15.205	245654	0.81
26	E-15-Heptadecenal	16.051	213853	0.71
27	Octadecane	16.209	231935	0.77
28	Nonadecane	18.529	151825	0.50
29	Hexadecane,1-iodo-	18.817	349997	1.16
30	2-Bromotetradecane	19.805	314972	1.04
31	n-Hexadecanoicacid	19.920	2108720	6.98
32	Hexadecanoicacid,ethylester	20.657	1818073	6.02
33	Octadecane	20.873	175967	0.58
34	2-Hydroxy-(Z)9-pentadecenylpropanoate	22.976	141881	0.47
35	Heneicosane	23.182	477794	1.58
36	Eicosane	23.695	355622	1.18
37	Linoelaidicacid	23.892	803867	2.66

38	9-Eicosenoicacid,(Z)-	24.033	1667334	5.52
39	11-Hydroxy-11-methyl-tricyclo[4.3.1.1(2,5)]u	24.130	282612	0.94
40	Linoleicacidethylester	24.513	5042943	16.70
41	(E)-9-Octadecenoicacidethylester	24.663	4533951	15.01
42	Hexadecanamide	24.909	97899	0.32
43	Octadecanoicacid,17-methyl-,methylester	25.255	595043	1.97
44	1-Tricosene	25.325	266936	0.88
45	Nonadecane	25.445	167625	0.56
46	Heptacosylacetate	25.591	166057	0.55
47	Hexadecanoicacid,2-hydroxy-1-(hydroxymet	31.998	248478	0.82
48	Di-n-octylphthalate	32.413	290173	0.96
49	Squalene	37.790	1026651	3.40
50	Stigmasterol	38.888	279326	0.92
			30198558	100.00

Table 2b. List of compounds in Methanol fraction of Crunchy kernel detected by GC/MS

S.No	Peak Name	Retention Time(min)	Area	Area%
1	N-Ethyl-N'-nitroguanidine	3.079	2981654	0.27
2	2-Propanone,1-hydroxy-	3.154	14043961	1.29
3	p-Dioxane-2,3-diol	3.229	9187575	0.84
4	2,2'-Bioxirane	3.566	3425784	0.31
5	Aceticacid,(acetyloxy)-	3.661	1967182	0.18
6	Aceticacid,methylester	3.700	7228833	0.66
7	Diethoxymethylacetate	3.761	12773483	1.17
8	Propanoic acid,2-oxo-,methylester	3.821	6706133	0.62
9	2,3-Butanediol,[R-(R*,R*)]-	3.924	8090005	0.74
10	(S)-5-Hydroxymethyl-2[5H]-furanone	3.963	3475382	0.32
11	Furfural	4.228	39313567	3.61
12	2-Furanmethanol	4.467	12591687	1.16
13	4-Penten-2-one,3-methyl-	4.531	4219125	0.39
14	(+-)-4-Amino-4,5-dihydro-2(3H)-furanone	4.740	14716308	1.35
15	Ethanone,1-(2-furanyl)-	4.945	1303285	0.12
16	2(5H)-Furanone	5.000	4946350	0.45
17	2-Cyclopenten-1-one,2-hydroxy-	5.148	8933974	0.82
18	3-Methyl-3-buten-1-ol,acetate	5.239	2820096	0.26
19	1H-Imidazole-4-carboxylicacid	5.321	804897	0.07
20	2-Furancarboxaldehyde,5-methyl-	5.427	11273237	1.04
21	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	5.610	9475780	0.87
22	Triethylenediamine	5.795	14377683	1.32
23	1,4-Butanediol	5.950	4606368	0.42
24	2-Methyl-3-oxobutyronitrile	6.040	4058631	0.37
25	1,5-Hexadien-3-ol,acetate	6.085	1530681	0.14
26	Benzeneacetaldehyde	6.130	2728539	0.25
27	2-Nonene	6.167	4644637	0.43
28	Furaneol	6.374	23949730	2.20
29	6,7-Dioxabicyclo[3.2.2]nonane	6.481	18018873	1.66
30	2(3H)-Furanone,5-methyl-	6.625	1666430	0.15
31	Undecane,6-methyl-	6.757	2353315	0.22
32	4H-Pyran-4-one,,3-dihydro-3,5-dihydroxy-6	7.111	64842116	5.96
33	4H-Pyran-4-one,3,5-dihydroxy-2-methyl-	7.362	3129726	0.29
34	5-Acetoxymethyl-2-furaldehyde	7.474	5866822	0.54

35	5-Hydroxymethylfurfural	7.994	605183228	55.62
36	5-Acetoxytridecane	8.400	11419522	1.05
37	1,3,2-Dioxaborolane,4,4-dimethyl-5-oxo-,2-e	8.638	18715273	1.72
38	Sulfoxide,butylpropyl	8.820	7196341	0.66
39	9-Imino-12-phenyl-10,11-dioxa-tricyclo[6.2.2.	8.930	3708196	0.34
40	Succinicacid,3-hex-4-ynyl3-methylbutylste	9.192	8528993	0.78
41	6-Oxa-bicyclo[3.1.0]hexan-3-ol	9.320	2073607	0.19
42	Isopropylphosphonicacid,dicylcopentylester	9.453	6496619	0.60
43	3-Bromo-5,5-dimethyl-cyclohex-2-enol	10.090	7894453	0.73
44	.beta.-D-Glucopyranose,1,6-anhydro-	10.663	43546394	4.00
45	n-Hexadecanoicacid	17.640	8802096	0.81
46	9-Oxabicyclo[6.1.0]non-6-en-2-one	18.375	16915165	1.55
47	Linoelaidicacid	21.296	3508597	0.32
48	cis-Vaccenicacid	21.426	5582435	0.51
49	Octadecanoicacid	21.919	3011270	0.28
50	OleicAcid	26.182	3519928	0.32
			1088153966	100.00

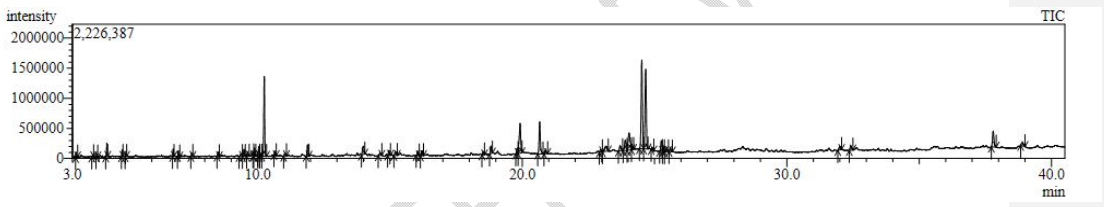

Fig 1a. GC/MS chromatogram of hexane extract of Crunchy kernel of *Borassusflabellifer L.*

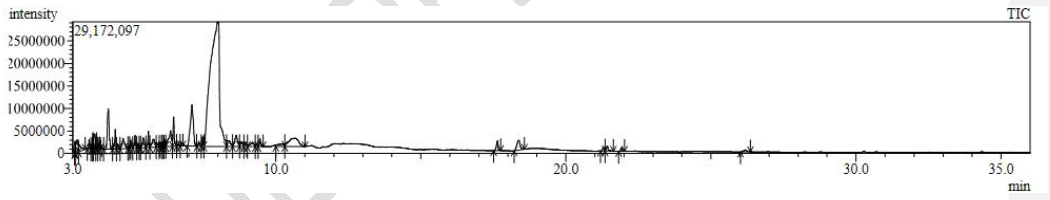

*Fig 1b.*GC/MS chromatogram of methanol extract of Crunchy kernel of *Borassusflabellifer L.*

From the results, it was observed that Linoleicacidethylester, (E)-9-Octadecenoicacidethylester, ButylatedHydroxytoluene are major compounds present major in hexane extract and 5-Hydroxymethylfurfural, beta.-D-Glucopyranose,1,6-anhydro-, 4H-Pyran-4-one,,3-dihydro-3,5-dihydroxy-6, Furfural are major compounds present in methanolic extract.

9–octadeceneic acid ethyl ester ($C_{20}H_{38}O_2$**)** is a fatty acid ester produced by the body during ethanol intoxication which could be used as a food additive or flavouring agent (22).Butylated hydroxytoluene (BHT)($C_{15}H_{24}O$**)** is a lipophilic organic compound, behaves as a synthetic analogue of vitamin E, chemically a derivative of phenol, that is useful for its antioxidant properties. BHT is listed under several categories in catalogues and databases, such as food additive,

household product ingredient, industrial additive, personal care product/cosmetic ingredient, pesticide ingredient, plastic/rubber ingredient and medical/ veterinary/ research (23). BHT is primarily used as an antioxidant food additive and also used as a preservative ingredient in some foods. With this usage BHT maintains freshness or prevents spoilage; it may be used to decrease the rate at which the texture, color, or flavor of food changes (24). BHT has also shown anti-viral activity (25, 26,27). Linoleic acid ethyl ester ($C_{20}H_{36}O_2$) lipid-soluble form of linoleic acid, essential fatty acid (poly unsaturated omega 6- fatty acid) which is used as anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties when applied topically on the skin. Its deficiencies lead to defective wound healing, growth retardation, and dermatitis.

Beta - D- Glucopyranose 1,6-anhydro (Levoglucosan) **(**$C_6H_{10}O_5$) is an organic compound with a six-carbon ring structure formed from the pyrolysis of carbohydrates, such as starch and cellulose. Levoglucosan is often used as a chemical tracer for biomass burning in atmospheric chemistry studies, particularly with respect to airborne particulate matter. This is because the gas emitted by the pyrolysis of wood (biomass) contains significant amounts of levoglucosan. Levoglucosan has been described as "an unequivocal biomass burning tracer" in the context of forest and brush fires. The hydrolysis of levoglucosan generates the fermentable sugar glucose. Levoglucosan can be utilized in the synthesis of chiral polymers such as unhydrolysable glucose polymers.Hydroxymethylfurfural (HMF) **(**$C_6H_6O_3$) is an organic compound formed by the dehydration of certain sugars, a major metabolite in humans is 5-hydroxymethyl-2-furoic acid (HMFA), which is excreted in urine. HMF bind intracellular sickle hemoglobin (HbS). Preliminary *in vivo* studies using transgenic sickle mice showed that orally administered 5HMF inhibits the formation of sickled cells in the blood(28). Under the development code Aes-103, HMF has been considered for the treatment of sickle cell disease(29).Furfural($C_5H_4O_2$**)** is an important renewable, non-petroleum based, chemical feedstock. It can be converted into a variety of solvents, polymers, fuels and other useful chemicals by a range of catalytic reductions (30). Furfuran resins can be produced from furfuryl alcohol (FA), which are exploited in thermoset polymer matrix composites, cements, adhesives, casting resins and coatings (31).

4. CONCLUSION

The present study was focused in two areas of enquiry. The first area revealed the antioxidant potential and it was found to be high in the crunchy kernel of *Borassusflabellifer.* In the second part of the study, characterisation of metabolites was done using GC/MS, which revealed the presence of various important compounds indicating that it may be used as a flavouring agent as well as has pharmacological significancein the promotion of health. The crunchy kernel is found to have possessed immense antioxidant potential and due to which it could be exploited effectively as value added food product.

Comment [µM12]: Author(s) should discuss which compounds in Table 2a are flavouring agent.

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Comment [µM13]: The format of each reference is not consistent. Please correct them thoroughly.

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Comment [µM14]: