Metabolomics analysis of *Celastruspaniculatus* seed extracts

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ABSTRACT

Celastruspaniculatus(CP), a traditional herb belongs to the family of *Celastraceae* known for its memory and cognitive enhancing properties. The CP seeds were the extensively studied material, but its metabolomics was still unknown particularly using combined extraction method. Hence the aim of this study is to determine the bioactive compounds of CP seeds using various solvents and by combined extraction method. CP seeds were procured from Kanyakumari, Tamilnadu and subjected to extract preparation with different solvents. After the initial phytochemical analysis, total and combined ethanolic extract of CP were chosen for further study. Gas chromatography-mass spectrometry (GC-MS) and Thin-layer chromatography (TLC) outcomes were analyzed to draw an inference based on variation in its metabolomics. A better understanding of its effectiveness against various medicinal conditions required further research.

Keywords: *Celastraceae*,Gas chromatography mass spectrometry,Metabolomics, Plant extracts; Solvents.

Introduction

Traditional medicines are used in the form of crude forms which are a mixture of various compounds and their effect on the biological system is still unexplained. Even trace amounts of some compounds in the extract can exert certain biological activity by itself or sometimes in a synergistic action with other components. If a principle active component is known by standardization, then analysis of this compound may contribute to the identification of its therapeutic efficacy.

Celastruspaniculatus(CP)is the member of the family Celastraceae and better known as Maalakaangni in Hindi, Vaaluluvai in Tamil and Jyotishmati in Sanskrit. It is widely distributed throughout the tropical region especially in Southeast Asia, the Caribbean Islands, the Pacific and Africa. *Celastruspaniculatus* is also referred to as the "elixir of life" in traditional medicine, due to its cognition enhancement properties (Godkar et al. 2004). Even though aerial parts of CPare known for its biological effects; its seeds have been extensively studied among others.

The prominent biological properties of the plantinclude nootropic effect (Bhanumathy et al. 2010), Antioxidant effect (Kumar et al. 2002), Analgesic & Anti-inflammatory effect (Afsahul et al. 2019), Antidepressant effect (Parimala et al. 2009). Further, the seeds of CP also studied for wound palliating properties, epilepsy, gouts, paralysis, and dysmenorrhea. The oil of its seed use to ameliorate amenorrhea, dermal disorders and dysentery, similarly the roots of CP are proven to attenuate scorpion venom (Valecha et al. 2016).

Many *in vivo* experimentalstudies have disclosed that the seeds of CP have cognitive strengthening effect on experimental animals, due to its high antioxidant potential (Neha et al. 2014). However, we lack studies that compared the efficacy of solvents used for isolation of seed extracts of CP. Hence the rationale is to evaluate the phytochemical analysis of extracts of the CP seeds prepared from low polarity solvent (petroleum ether), mild polarity

(Chloroform), and moderate polarity (Ethanol) to high polarity (Water) in addition to these extracts, combined ethanolic extract was also studied. The comparison of TLC and GC-MS investigation of total and combined ethanolic seed extract of CP were done to know the difference in the compounds present in it.

Materials and methods

Procurement of materials

The chemicals and solvents were molecular and analytic grade, acquired from SISCO research laboratories Pvt Ltd, DK enterprises, India and Sigma Aldrich, USA. The seeds of CPwere obtained from Kanyakumari district of Tamilnadu during the evening of a sunny day of June 2018 with proper authentication. The seeds were transported to the lab cleaned it to remove undesirable compounds, subjected to shadow drying and extraction was performed using standard procedure.

Extract preparation

Dry, coarse powderof plantseed material of 150g was soaked in 250mL of 90% ethanol in aspirator bottle. After 72 h, the mixture was put through the soxhlet extraction procedure for 18 h. The final traces of solvent were removed using a rotary evaporator. The yield from the extraction was about 20%. In a similar way, the preparation of extract from petroleum ether, hexane, chloroform and distilled water was done. The aqueous extract was placed in a water bath at 50°C in order to get concentration. For the preparation of combined extract, seed material immersed in the petroleum ether for 72 h was reused to prepare extract with ethanol after filtering it.

Preliminary phytochemical analysis

The phytochemical tests were done to find the various substances present in the CP seed extract based on the methods suggested by (Harborne JB 1983; Agrawal PK *et al*., 1987; and Sazada S *et al*., 2009).

Thin-layer chromatography

Thin-layer chromatography was done using mobile phase (n-Hexane: Ethyl Acetate (9:1, v/v), (8:2, v/v), Toluene: Ethyl Acetate: Glacial acetic acid (6:1.5:0.1, v/v), Ethyl Acetate: Methanol (4:6, v/v)) and the Stationary Phase: (Readymade TLC plate Merck Silica gel GF254 (20*20cm)). The plates were applied with Silica gel and build to a homogenous suspension with distilled water (60 mL), this suspension was disseminated on the plate which was arid up to the fading of layer's transparency. The plates were kept and dried in the oven at 120°C for 25 minutes and then stocked in a desiccated place. Samples were applied to the launch end of a thin layer chromatography plate few centimeters above its lower end by utilizing capillary tubes. After the administration of the sample, the plates were mounted in the thin-layer chromatography chamber than the mobile phase was admitted to proceed through the adsorbent phase until predominant part of the plate (Kannan, 2010 &Jing, 2013). The reactive oxygen species scavenging properties of the CP seed extracts were evaluated by the procedure with DPPH (2, 2-diphenyl picryl hydrazyl). The sample was spotted and eluted in difference mobile phases. The TLC plates were sprayed with DPPH and Fade yellow colour in purple background was recorded.

Gas chromatography - mass spectrometry

The gas chromatography-mass spectrometry (GC-MS) investigation was done using following Instrument: GC (Agilent: GC: [G3440A] 7890A. MS MS: 7000 Triple Quad GCMS) was armed with a mass spectrometry detector. The following procedure was used: Column DB5 MS (30 mm × 0.25 mm ID × 0.25 μm, composed of 5% phenyl 95% methyl polysiloxane); electron impact mode at 70 eV; helium (the carrier gas) was applied at a sustained rate of 1 mL/minute source injector temperature 270°C; Auxilary temperature was kept at 280°C, and the ionization temperature was switched to 270°C. The instrumental oven temperature was set between 40°C (isothermal for 1.0 minute), with a steady rise of

30°C/minute, upto 160°C (isothermal for 4.0 minutes), and then rise of 10°C/minutes for until 300°C (isothermal for 10 minutes) fragments ranging between 45 - 450 Da. The total gas chromatography running time is 32.02 minutes. The chemical compounds were recognized by comparing with GC–MS Library (National Institute of Standards and Technology and Wiley).

Result and discussion

Preliminary phytochemical investigation done for various extracts of seeds of CP suggested the presence of flavonoids, phenolic compound, alkaloids, saponins, steroids, tannin, terpenoids, amino acids, carbohydrates, cumarines, fixed oil, glycosides (Table 1).

Table 1. Phytochemical analysis of various extracts of *Celastruspaniculatus* seeds.

S N	Phytochemical	Test name	Results of various extracts of CPSeeds				
			Petroleum Ether	Chloro-form	Total Ethanolic	Combined Ethanolic	Water
1	Carbohydrate	Benedict's Test	-	+	+	+	+
2	Protein & Amino Acids	Millons Test	-	-	+	+	-
3	Alkaloid	Dragendorff's Test	-	+	+	+	+
4	Tannin & Phenol	Ferric Chloride Test	-	+	-	-	+
5	Flavonoids	Zn – HCl Test	-	-	+	+	-
6	Steroid / Terpenoids	Salkowski Test	-	-	+	+	+
7	Saponin	Froth Test	-	-	-	-	+
8	Glycosides	Keller-Kilani Test	-	-	+	+	-

9	Quinons	NaOH Test	-	-	-	--	--
10	Fixed Oil	Paper/spot Test	+	+	+	+	+
11	Resins	Acetone Test	-	-	+	+	+
12	Coumarins	Fluorescence Test	+	-	-	-	-
13	Carotenoids	-	-	-	-	-	-

The information obtained from the preliminary phytochemical screening was used to find the extract with the highest yield. Total and combined ethanolic extract was selected for further investigations. And it was noted that petroleum ether extract of CP seeds showed the presences of fixed oil and coumarins. Hence the combined ethanolic extract of CP seeds has proven to retain all the bioactive compounds which have beneficial biological activities (Table 2).

Table 2. TLC screening of Total and Combined ethanolic extract of CP Seeds

S N	Phytochemical	Reagent sprayed	Appearance	Result	
				Total Ethanolic Extract	Combined Ethanolic Extract
1	Phytochemicals	UV-254nm	Purple colour	+	+
2	Phytochemicals	UV-365nm	Florescent colour	+	+
3	Phytochemicals	Iodine	Brown colour	+	+
4	Phenols/Tannin	10%Ferric Chloride	Black/Green colour	-	-
5	Amino acids	Ninhydrin reagent	Multi colours	-	-
6	Phytochemicals	H2SO4-Methanol	Multi colours	+	+
7	Terpenoids	Vanillin-H2SO4	Multi colours	+	+
8	Alkaloids	Dragendorff's	Reddish orange	-	+
9	Flavinoids	AlCl3	Fluorescent green	+	+

10	Flavinoids/Quinones	10% KOH	Fluorescent green	-	-
11	Phyto/Terpenoids	Anisaldehyde	Multi colours	-	+
12	Flavinoids	Lead Acetate	Fluorescent green	+	+

The TLC plates were sprayed with DPPH reagent showed fade yellow colour in purple background indicating antioxidant activity (Figure 1).

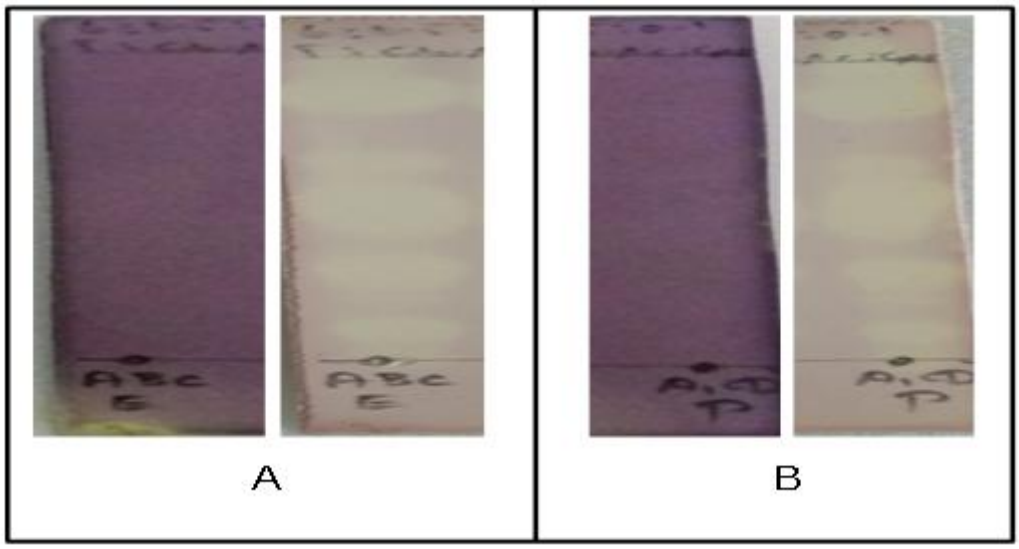

Figure 1. TLC analysis of antioxidant activity DPPH reagent, A – Total ethanolic extract of CP seeds, B – Combined ethanolic extract of CP seeds.

The details of GC–MS profile oftotal and combined ethanolic extract of*Celastruspaniculatus*seeds are shown in Table 3 and Table 4 respectively.

GC–MS profile of total ethanolic extract of *Celastruspaniculatus* seeds showing various molecules present in it with its retention time.

Retention Time	Name of molecules	Formula	Area	Height	Mass
18.05	Hexadecanoic acid, ethyl ester	C18H36O2	2115744512.00	8,01,97, 73,952	284
18.955	N-Hexadecanoic	C16H32O2	191852928.00	2,40,24,	256

	acid			16,896	
19.2	9-Octadecenoic acid, ethyl ester	C20H38O2	3373765376.00	28,60,71,62,368	310
19.415	9,12,15-Octadecatrienoic acid, ethyl ester, (z,z,z)-	C20H34O2	1924456320.00	12,77,80,50,560	306
20.371	1,19-Eicosadiene	C20H38	1637344640.00	2,83,57,86,752	278
21.101	1-Hexyl-2-Nitrocyclohexane	C12H23O2N	403968704.00	1,56,42,60,480	213
23.952	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (all-E)-	C30H50	189157472.00	2,51,05,64,096	410
24.157	Acetic acid, 1-[2-(2,2,6-Trimethyl – Bicyclo [4.1.0] Hept-1-yl) - ethyl] - Vinyl ester	C16H26O2	289243360.00	3,08,76,05,504	250
25.137	4-hydroxy-5,6-epoxy-.beta.-ionone	C13H20O3	890140864.00	13,36,24,15,616	224
25.332	1-(5-Methyl-2-oxy-[1,2,4]Oxadiazol-3-yl) – 2 – Phenyl – Ethane - 1, 2 -dione 1-oxime	C11H9O4N3	270340736.00	4,31,81,78,304	247
25.433	1,3-Butanedione, 2-Methyl – 1 - Phenyl	C11H9O4N3	178112560.00	2,92,84,72,832	247
25.893	1,2,3,4 - Butanetetrol, 2,3-Diacetate 1 , 4 - Dibenzoate, (R*,S*)-	C22H22O8	317993664.00	3,85,17,20,704	414
26.133	2,2,3-Trimethyl-1-Phenyl-3 – Buten – 1 – one	C32H44O6	138450512.00	1,92,34,41,408	524

Table 4. GC–MS profile of combined ethanolic extract of *Celastruspaniculatus* seeds showing various molecules present in it with its retention time.

Retention Time	Name of molecules	Formula	Area	Height	Mass

18.37	Hexadecanoic acid, ethyl ester	C18H36O2	615540736.00	3,01,59,44,192	284
19.13	N-Hexadecanoic acid	C16H32O2	362998560.00	2,30,72,99,072	256
19.325	9 - Octadecenoic acid, ethyl ester	C20H38O2	1549358208.00	16,38,80,27,392	310
19.54	Dichloroacetic acid, Tridec – 2 - ynyl ester	C15H24O2Cl2	863905536.00	5,34,97,22,624	306
20.375	18 – Nonadecen – 1 - ol	C19H38O	1308468352.00	2,62,31,88,736	282
24.187	Cis, cis-2,9 – Dimethylspiro [5.5] undecane	C13H24	205226192.00	2,13,90,45,504	180
25.197	5 - Isopropyl-6, 6 – Dimethyl Hept – 3 – yne - 2, 5 - diol	C28H38O9	583350976.00	7,15,18,84,800	518
25.397	Carpesterol dehydrate	C37H52O3	196975616.00	2,53,13,12,384	544
25.738	Cyclohexanemethanol, 4-(1-methyl ethyl)-, Transho	C13H20O3	118045288.00	2,02,74,42,048	156
25.868	Pregn-4-ene-3, 20 - Dione, 11- Hydroxy-, (11.alpha.) -	C21H30O3	141068000.00	2,08,25,45,664	330
25.953	1,2,3,4 - Butanetetrol, 2,3 - Diacetate 1,4-Dibenzoate, (R*, S*)-	C22H22O8	33,29,99,200	4111994368.00	414
26.163	Benzoic acid, 2 – Fluoro- , 2 – oxo – 2 – Phenyl ethyl ester	C15H11O3F	99491752.00	1,54,39,76,704	258
26.458	Cholesteryl Benzoate	C37H52O3	85304480.00	1,33,90,84,288	544
27.088	7 - DehydrocholesterylIsocaproate	C33H54O2	74828160.00	89,94,48,768	482

Figure 2 and Figure 3 shows the GC–MS chart representing the numerous peaks analogous to each chemical compound present in total and combined ethanolic extracts of *Celastruspaniculatus*seeds respectively.

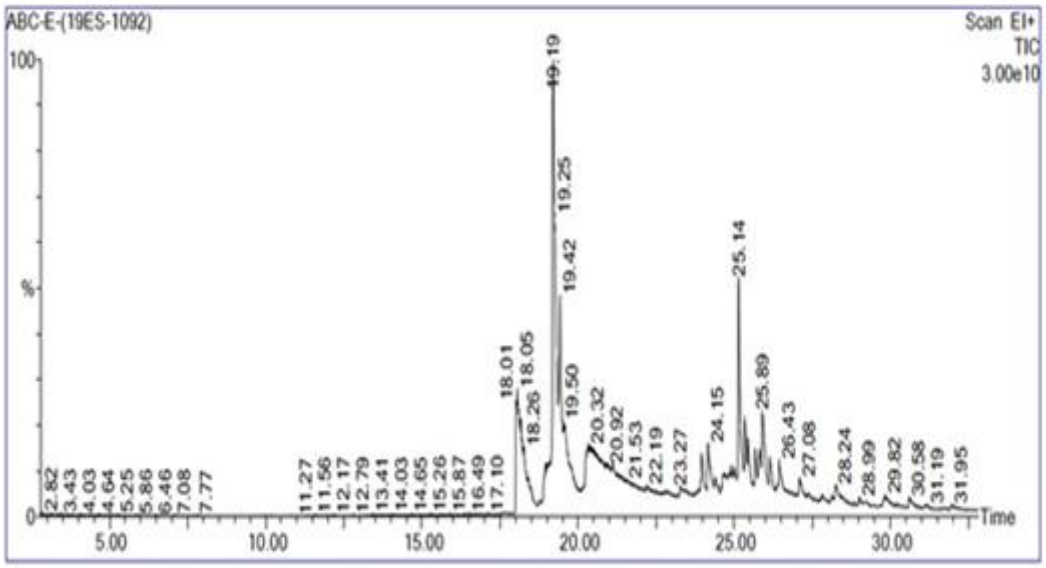

Figure 2. Indicates the GC–MS graph representing the various peaks corresponding to each molecule present in total ethanolic extract of *Celastruspaniculatus*seeds.

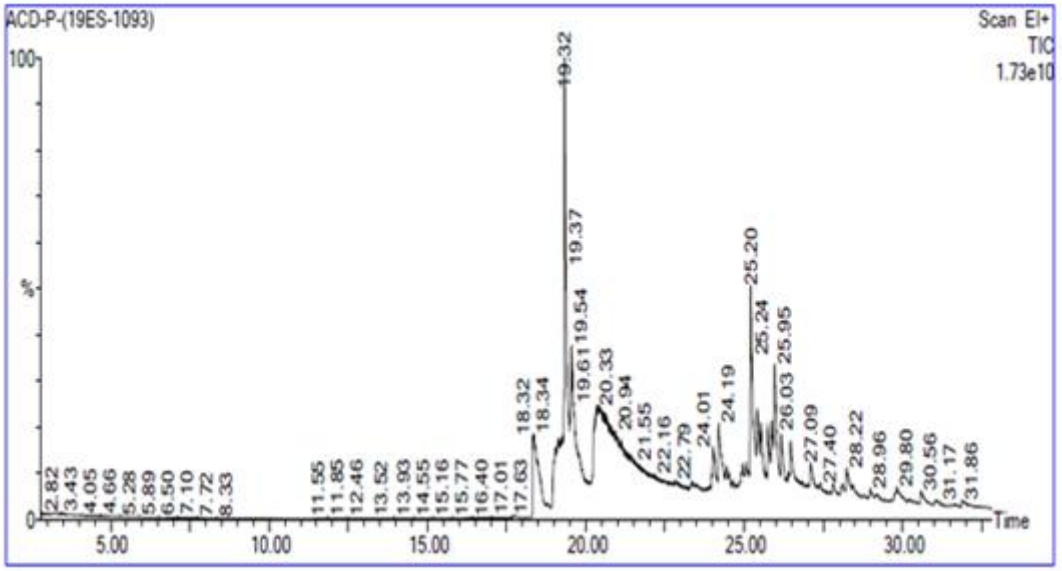

Figure 3. Indicates the GC–MS graph representing the various peaks corresponding to each molecule present in combined ethanolic extract of *Celastruspaniculatus*seeds.

The floral sphere represents a vast amount of bioactive molecules and to date only a fragment of it with remedial capacity has been analyzed. Just about half of the drugs used in the field of medicine are originate from herbs. Hence recent researches dedicated to the qualitative and quantitative phytochemical analysis of plants which have ethno-botanical details connected with them. The phytochemical studies and analysis help in determining the chemical structures, origin of biosynthesis and mechanism of action of natural hormones. Another rationale for phytochemical investigation is the characterization of an active principle accountable for few toxic or beneficial effects shown by a crude extract of plant when studied against an in-vivo condition. On such occasions, it is vital to observe the extraction and separation procedures at each stage in order to follow the bioactive compound as it is purified. The chance of damage to the bioactive compound during isolation and identification, characterization must consistently be borne in mind. On the other hand, crude extracts can be assayed for specific activities and the active fractions investigated phytochemically (Hartmann et al. 2007, Kimura et al. 2001).

The plants have secondary metabolites as compared with primary metabolites such as proteins, carbohydrates and lipids. The biological roles of these compounds are poorly understood, whatever bioactive compounds studied so far are used as pharmaceuticals, insecticide, artificial flavoring of eatables and as ingredients in cosmetics (Ramadan et al. 2010). The total and combined ethanolic extracts of seeds of CP are rich in palmitic acid, squalene, ethyl linolenate, fatty acids, ketones, and esters (Table III & VI). Apart from these compounds, both the extracts also contain flavonoids, alkaloids and terpene compounds. Literature reviews showed that palmitic acid, a form of vitamin C is also an antioxidant

which is proven to be more effective antioxidant (Sengupta et al. 2006, Madhavi et al. 2005). From the previous reports, it was found that palmitic acid has anti-androgenic (Tyagi et al. 2017), antioxidant (Gavamukulya et al. 2015), hypo-cholesterolemic (Aparna et al. 2012), anti-inflammatory (Kumar et al. 2010), antimicrobial (Selvamangai et al. 2012), nematicide and mosquito larvicidal properties (Perumalsamy et al. 2013 &Agoramoorthy et al. 2007).

Many phenolic and alcoholic compounds have been identified in the extracts of both solvents. 1,3-Butanedione, 2-methyl-1-phenyl, 2,2,3-Trimethyl-1-phenyl-3-buten-1-one and more specifically 1-(5-methyl-2-oxy-[1,2,4]oxadiazol-3-yl)-2-phenyl-ethane-1,2-dione 1-oxime which was found in the total extract of CP seeds possess antioxidant potential (Sandeep et al. 2014 &Ercan et al. 2021). Ethyl linolenate has therapeutic potential in reducing oxidative damages thereby it found to possess the cognition improving, neuroprotective activities, hepatoprotective, antihistaminic, antiandrogenic, and antiarthritic effects (Bharathy et al. 2012, Devanesan et al. 2021, Rahuman et al. 2000).In the present study, the GCMS profile of CP seeds revealed major constituents which are well known to naturally have distinguishable powerful antioxidant properties. 1-Hexyl-2-Nitrocyclohexane, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)- were known for their anti-inflammatory, analgesic property, and antioxidant, antibacterial, immunostimulant, and chemo preventive (Chitrali et al. 2018, Thirumalaisamy et al. 2018, Dinesh et al. 2014, Sudha et al. 2013).

The variation in temperature, precipitation, soil moisture and fertility are the factors that affect the levels of phytochemicals, antioxidants and secondary metabolites present in plants. Hence it is always better to compare the phytochemical investigation of plant materials collected from the same geographical location and in the same period of the year (Kumar et al. 2017, Ramazan et al. 2021). Intracellular secondary metabolites are contained within a mechanical barrier, the cell membrane. Hence, in order to identify and quantify these

secondary metabolites, a proper method of extraction should be used to obtain the intracellular secondary metabolites. Therefore, petroleum ether was used to break the mechanical barrier, the cell wall which is made of lipid molecules. Later the intracellular secondary metabolites were extracted using 90% ethanol. The membrane permeability was altered through the application of petroleum ether followed by 90% ethanol. This method helped to identify ten different compounds in the *combined* ethanolic extract from *that of total ethanolic extract* of CP seeds using GC-MS profile (Table VI). Bioactive compounds such as Dichloroacetic Acid - Tridec-2-ynyl Ester, 18-Nonadecen-1-ol, Cis,cis - 2,9- dimethyl spiro [5.5] undecane, 5 – isopropyl – 6 , 6 – dimethyl hept – 3 – yne – 2 , 5 - diol, Carpesterol dehydrate, Cyclohexane methanol 4-(1-methyl ethyl)- transho, Pregn – 4 – ene - 3 20 - dione, 11 – hydroxyl - (11.alpha.)-, Benzoic acid, 2 – fluoro – 2 – oxo – 2 – phenyl ethyl ester, Cholesteryl benzoate and 7-dehydrocholesteryl isocaproate are found in combined ethanolic extract of CP seeds. Among these, two new compounds which are not found in the PubChem compounds database, Cis, cis – 2, 9 –dimethylspiro [5.5] undecane, Cyclohexane methanol 4-(1-methyl ethyl)- transho were found in the combined ethanolic extract of CP seeds (Table VI). Thus, of the two studied extracts, combined ethanolic extract of CP seeds to have key bioactive compounds which have various biological activities as compared to total ethanolic extract of CP Seeds.

Conclusion

GC–MS investigation of the total and combined ethanolic extract of CP seeds suggests that they are abundant in antioxidant compounds and have free radical scavenging properties to reduce oxidative stress. The bioactive compounds of both the extracts of CP seeds, such as, palmitic acid, ethyl linolenate and 1-(5-methyl-2-oxy-[1,2,4]oxadiazol-3-yl)-2-phenyl-ethane-1,2-dione 1-oxime are found to be genuine antioxidants that possess possible parts in eliminating reactive oxygen species and attenuate oxidative stress. CP is a potential

herbal plant and its seeds are abundant in natural antioxidant compounds and numerous beneficial medicinal properties. This indicates that the combined ethanolic extract of seeds of the CP could have a potential capacity in oxidative stress associate diseases and the newly identified bioactive compounds specifically Cis, cis – 2, 9 –dimethyl spiro [5.5] undecane, Cyclohexane methanol 4-(1-methyl ethyl)- transho, in CP seeds could be used as a prospective beginning for new medicine occurrence for numerous diseases related to oxidative stress.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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References

Afsahul, K., Suheena, K., Sajjad, S. and Malkangni, A. (2019). *Celastrus paniculatus* wild: neuropharmacological properties in the perspective of Unani medicine and pharmacological studies - A Review. World journal of pharmacy and pharmaceutical sciences, 08: 381-391.

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V. and Hsu, M.J. (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol, 38(4): 739-42.

Agrawal, P.K., Daldlani, M. and Shrava, I. (1987). Technique in seed science and technology.

India Agriculture Research Institute, 11: 107-112.

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. (2012). Anti-inflammatory property of n-Hexadecanoic acid: Structural evidence and kinetic assessment. Chem. Biol. Drug Des, 80: 434–439.

Bhanumathy, M., Harish, M.S., Shivaprasad, H.N. and G. Sushma. (2010). Nootropic activity of Celastrus paniculatus seed, Pharmaceutical Biology, 48: 3, 324-327.

Bharathy, V.B., Sumathy, M. and Uthayakumari, F. (2012). Determination of phytocomponents by GC – MS in leaves of *Jatropha gossypifolia* L, Sci. res. report, 2(3): 286-290.

Chitrali, R., Sathyamurthy, A. and Balasubramanian. (2018). GCMS and FTIR analysis on the methanolic extract of red Vitis vinifera peel. WJPPS, 8: 12148.

Devanesan, S., Jayamala, M., AlSalhi, M.S., Umamaheshwari, S., Ranjitsingh, A.J.A. (2021). Antimicrobial and anticancer properties of Carica papaya leaves derived di-methyl flubendazole mediated silver nanoparticles. J Infect Public Health, 14(5):577-587.

Dinesh, M.G., Subbarayan, R., Rallapalli, S., Kansrajh, C. and Kalaivani, R. (2014). Terminalia bellirica leaf extracts induce apoptosis in Hep G2 cells and regulates cell cycle progression by inducing G2/M cell cycle arrest, Indian. J. Res. Pharm. Biotechnol, 2(1): 1044-1056.

Ercan, K., Gecesefa, O.F., Taysi, M.E., Ali, O.A., Taysi, S. (2021). Moringa Oleifera: A Review of Its Occurrence, Pharmacological Importance and Oxidative Stress. Mini Rev Med Chem, 21(3):380-396.

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F. and El-Shemy, H.A. (2015). GC-MS

Analysis of Bioactive Phytochemicals Present in Ethanolic Extracts of Leaves of Annona muricata: A Further Evidence for Its Medicinal Diversity. Pharmacognosy Journal, 7(5): 300-4.

Godkar, P.B., Gordon, R.K., Ravindran, A. and Doctor, B.P. (2004). *Celastrus paniculatus* seed water-soluble extracts protect against glutamate toxicity in neuronal cultures from rat forebrain. J. Ethnopharmacol, 93, 213-219.

Harborne, J.B. (1983) Phytochemical Method: A guide to Modern Techniques of Plants Analysis. 2nd Edn. Chapman and Hall New York,

Hartmann, T. (2007). From waste products to eco chemicals fifty years research of plant secondary metabolism. Phytochemistry, 68(22–24): 2831–2846.

Jing, R.W., Ming, H.Y. and Wei, Y.L. (2013). Cytotoxic constituents from *Celastruspaniculatus* induce apoptosis and autophagy in breast cancer cells. Photochemistry, 94: 211-9.

Kimura, M., Anzai, H. and Yamaguchi, I. (2001). Microbial toxins in plant-pathogen interactions: Biosynthesis, resistance mechanisms, and significance. J. Gen. Appl. Microbiol, 47(4): 149–160.

Kannan, R., Arumugam, R. and Meenakshi, S. (2010). Thin layer chromatography analysis of antioxidant constituents from sea grasses of Gulf of Mannar, Biospiosphere Reserve, South India. Int. J. Chem. Tech Res, 2: 1526-1530.

Kumar, M.H.V. and Gupta, Yogendra. (2002). The antioxidant property of *Celastrus paniculatus* Willd: A possible mechanism in enhancing cognition. Phytomedicine: international journal of phytotherapy and phytopharmacology, 9, 302-11.

Kumar, P.P., Kumaravel, S. and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr. J. Biochem. Res, 4: 191–195.

Kumar, S., Yadav, A., Yadav, M. and Yadav, J.P. (2017). Effect of climate change on

phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f. BMC. Res. Notes, 10(1): 60.

Madhavi, D.L., Deshpande, S.S. and Salunkhe, D.K. (2005). Food Antioxidants Technological:

Toxicological and Health Perspectives, 205–209.

Neha, A., and Shashi, P.R. (2014) GC–MS analysis of the essential oil of

Celastrus paniculatus Willd. Seeds and antioxidant, an anti-inflammatory study of its various solvent extracts, Ind. Crops Prod, 61: 345-51.

Parimala, S., Shashidhar, G.H., Sridevi, C., Jyothi, V. and Suthakaran, R. (2009). Anti-inflammatory activity of *Celastruspaniculatus* seeds. International Journal of PharmTech Research, 1(4): 974-4304.

Perumalsamy, H., Jang, M., Kim, J.R., Murugan, K. and Ahn, Y.J. (2015) Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettiapinnata* seed toward three mosquito species. Parasites and Vectors, 8(1):237.

Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S. and Himalayan, B. (2000).

Effect of Feronia limonia on mosquito larvae. Fitoterapia, 71: 553–555.

Ramadan, M.F. (2010). Lipids of *Celastrus paniculatus* seed oil. Chem. Nat, 46, 4.

Ramazan, G., İbrahim K. (2021) Possibility of using leaf extracts of tree-of-heaven (Ailanthus

altissima (Mill.) Swingle) for the postharvest quality preservation of fresh apricot fruits. Physiol. Mol. Plant Pathol. 113.

Sandeep, D., Seema, D., Sonam, R., Meenakshi, B., Vinay, M. and Anil, K. (2014). Isolation

and Biological Evaluation of Novel Tetracosahexaene Hexamethyl, an Acyclic Triterpenoids Derivatives and Antioxidant from *Justicia adhatoda*. CComb. Chem. High Throughput Screen, 17: 723.

Sazada, S., Verma, A., Rather, A., Jabeen, F. and Meghvansi, M. (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. in Biol. Res, 3: 188-195.

Selvamangai, G. and Bhaskar, A. (2012). GC–MS analysis of phytocomponents in the methanolic extract of Eupatorium triplinerve. Asian Pac. J. Trop. Biomed, 2(3): 1329-32.

Sengupta, A. and Bhargava, H.N. (2006). Chemical investigation of the seed fat of Celastruspaniculatus. J. Sci. Food Agric, 21 (12): 628–631.

Sudha, T., Chidambarampillai, S. and Mohan, V.R. (2013). GC-MS Analysis of Bioactive Components of Aerial parts of FluggealeucopyrusWilld. (Euphorbiaceae). J. App. Pharm. Sci, 3 (05): 126-130.

Thirumalaisamy, R., Ammashi, S., Muthusamy, G. (2018). Screening of anti-inflammatory phytocompounds from Cratevaadansonii leaf extracts and its validation by in silico modeling. J Genet EngBiotechnol, 16(2):711-719.

Tyagi, T. and Mala, Agarwal. (2017). Phytochemical screening and GCMS analysis of bioactive constituents in the ethanolic extract of Pista stratiotes L and Eichhorniacrassipes (Mart.) solms, Journal of Pharmacognosy and Phytochemistry, 6(1): 195-206.

Valecha, R. and Dinesh, D. (2016). Behavioral and Biochemical Evidences for Antidepressant

Like Activity of *Celastrus Paniculatus* Seed Oil in Mice. Basic and clinical neuroscience, 7: 49-56.