Asian Journal of Chemistry; Vol. 26, No. 12 (2014), 3745-3748

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17067

LC-MSMS and GC-MS Analysis in Identification of Antioxidant and Immuno-Modulatory Molecules in *Amukkara curanam*†

K.P. Arun, V. Sudha and P. Brindha*

Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur-613 401, India

*Corresponding author: E-mail: brindha@carism.sastra.edu

Published online: 5 June 2014; AJC-15338

Amukkara curanam an anti HIV Siddha formulation was selected for the present work with a view to identify bioactive molecules that might be responsible in improving the immunity in HIV patients. This preparation is often prescribed by Siddha practitioners to manage this disorder. The selected formulation is procured from SKM Siddha Pharmacy (GMP certified), Erode, which contains eight different herbal ingredients. Attempts were being made to identify the bio active constituents present in the selected drug through GC-MS and LC-MSMS analysis. This is the first report on the chemical analysis of *Amukkara curanam* using sophisticated instrumentation techniques. The study revealed presence of antioxidant molecules such as furanone, caryophyllene oxide, α-zingiberene, zingiberone, apiol, α-eudesmol, nerolidol and gingerol. Besides, piperic acid an antioxidant and anti-viral molecule was also detected. Bio-enhancer and immunomodulatory agents such as piperine, withaferin A and withanolide A were also identified. Present study can contribute in providing chemical evidences for usage of this traditional preparation in the management of HIV, which helps the patients in enhancing their immune system.

Keywords: *Amukkara curanam*, LC-MSMS, GC-MS, Bioactive molecules.

INTRODUCTION

Herbal medicines are now resorted to as the best alternative system of medicines by the majority of world population. But the greatest lacuna existing in Traditional Indian herbal medicines are validation and standardization. In the present paper, a traditional Siddha preparation *Amukkara curanam*[1] which is often prescribed by Siddha practitioners in immune deficiency disorder such as AIDS is selected and evaluated using sophisticated instrumentation techniques such as LC-MSMS and GC-MS to provide supporting chemical evidences for its the rapeutic claims and in determining chemical standards, which can contribute significantly in establishing quality of Siddha preparations and making the system an internationally acceptable and globally recognizable.

Present work deals with developing methods for the chemical standardization of *Amukkara curanam*, a poly herbal siddha formulations constituting of eight herbal plants. With this view an attempt is also being made to standardize and to confirm the type of active molecules present in the formulation through LC-MSMS and GC-MS analysis.

EXPERIMENTAL

GC-MS analysis: GC analysis was carried out on a Clarus 500 gas chromatograph using a non-polar, Elite-5ms column (30 m × 0.25 mm × 0.25 µm film thickness, coated with 5 % diphenyl-95 % dimethyl polysiloxane) interfaced with mass detector. Helium was used as carrier gas with a flow rate of 1 mL/min. Temperature programme was 50-150 ºC hold for 2 min at the rate of 3 ºC/min and increased to 290 ºC (10 min) at the rate of 8ºC/min. 1 µL of the methanol extract was injected with split ratio as1:10. Mass spectra were recorded in the EI mode at 70 eV in a scan range of 40-600. Injector and ion source temperature were maintained at 280 and 200 ºC, respectively. The resulted spectrum was compared with NIST library database.

LCMS-MS analysis: Polar and semi polar molecules of aqueous extract of *Amukkarac curanam* was separated and identified using LC-MSMS. 1 mg/mL extract was chromatographed over C_{18} RP column (Acclaim 120 Å, 2.1 mm × 150 mm, 3.0 µm, Dionex, USA). Eluted compounds were then identified using MS and their respective MSMS pattern. UHPLC was conditioned at 0.2 mL/min flow rate, with gradient

†Presented at PHYTOCONGRESS-2013, held on 7-8 March 2013, SASTRA University, Thanjavur, India

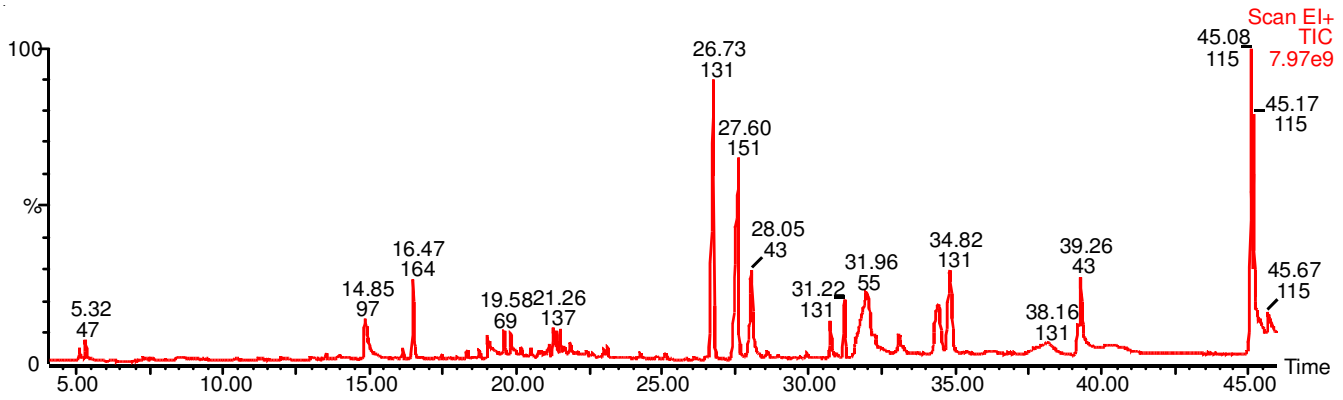

Fig. 1. GC-MS total ion chromatogram of *Amukkara curanam*

mobile system start at 1 % acetonitrile for 0.2 min and 99 % water (1 % acetic acid). This was then brought to 75 % acetonitrile at 16th min and then reaching at 100 % acetonitrile at 19th min to 5 % acetonitrile at 21st min and was maintained at same condition till run ends at 23rd min. Absorbance was read arbitrary at 325 nm. Exact mass of each eluted compound and their fragmented pattern (MSMS) were identified using ESI-Q-II TOF (Bruker, Germany) at negative mode, nebulizer was set at 30.5 psi with 6.0 L/min N_2 flow rate. Masses were analyzed in 50-1000 *m/z* range, keeping capillary voltage of 4500 V with dry heater temperature at 280 ºC.

RESULTS AND DISCUSSION

The ingredients used in the selected Siddha preparation is presented in Table-1.

TABLE-1
INGREDIENTS OF *Amukkarac curanam*

Plant/drug name	Scientific/common name	Quantity (g)
Nattu Amukkara. Kizhangu	*Withania somnifera*	640
Cukku	*Zingiber officinale*	320
Tippili	*Piper nigrum*	160
Milaku	*Piper longum*	80
Elakkai	*Eletteria cardamom*	40
Sirunagapoo	*Mesuafera*	20
Kirambu	*Syzygium aromaticum*	10
Carkkarai	*Saccharum officinarum*	1280

GC-MS analysis: GCMS data presented in Table-2 shows the presence of 26 fragmented compounds of which phenols, flavonoid and sugar moieties were found to be higher. The fragmented pattern of different compounds present in the *Amukkarac curanam* were detected and identified based on their fragment pattern available in NIST library. Furanone[2], caryophyllene oxide[2], α-zingiberene[3], apiol[4], α-eudesmol[5], nerolidol[6], gingerol[7] and piperic acid[8] are the important bioactive molecules identified and they are scientifically proven for their antioxidant potentials. GC-MS chromatogram of the methanolic extract was shown in Fig. 1 and their respective structures were shown in Fig. 2.

LC-MSMS analysis: LC-MSMS analysis helped in identifying glycowithanolides (Fig. 3) such as withanolide A and steroidal lactones like withaferins A, which are proven immuno modulatory and antioxidant agents[9,10]. Piperine an alkaloid with rich bioenhancing and antioxidant potential was also identified[11,12].

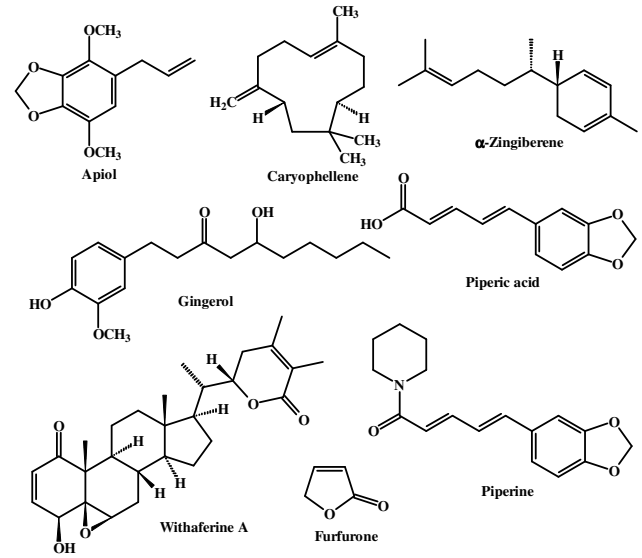

Fig. 2. Various antioxidant molecules identified in *Amukkara curanam* through GC-MS

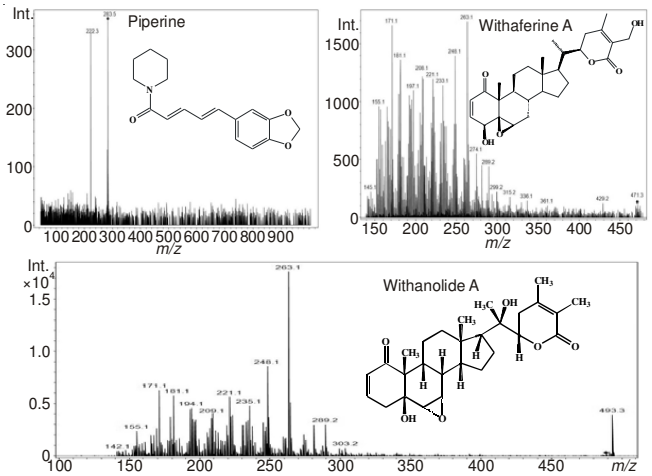

Fig. 3. MSMS fragmentation pattern of different phytoconstituents present in *Amukkarac curanam*

Conclusion

From the study it is concluded that major chemical constituents identified must have been derived from the major plant ingredients used in the formulation such as withanolides and withaferins from *Withania somnifera,* zingiberene and

TABLE-2
LIST OF FRAGMENTED COMPOUNDS

Peak name	Retention time	Peak area	Peak area (%)
Name: Hexane, 1-(ethenyloxy), m.f.: $C_8H_{16}O$, m.w.: 128	5.09	14937520	0.3917
Name: Furfural, m.f.: C_5H_4O2, m.w.: 96	6.11	3511483	0.0921
Name: 4,5-Dihydro-2-methylimidazole-4-one, m.f.: $C_4H_6N_2O$, m.w.: 98	8.45	27283022	0.7154
Name: 4-Octanol, 7-methyl-, m.f.: $C_9H_{20}O$, m.w.: 144	9.12	2023926	0.0531
Name: Octanal, m.f.: $C_8H_{16}O$, m.w.: 128	9.42	1774075	0.0465
Name: 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone, m.f.: $C_6H_8O_3$, m.w.: 128	11.53	891244	0.0234
Name: Phenylethyl alcohol, m.f.: $C_8H_{10}O$, m.w.: 122	12.21	1071588	0.0281
Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, m.f.: $C_6H_8O_4$, m.w.: 144	13.02	10391134	0.2725
Name: Decanal, m.f.: $C_{10}H_{20}O$, m.w.: 156	13.49	7272088	0.1907
Name: Glycerin, m.f.: $C_3H_8O_3$, m.w.: 92	14.01	13342372	0.3499
Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, m.f.: $C_6H_6O_3$, m.w.: 126	14.85	107761416	2.8257
Name: 2-Methoxy-4-vinylphenol, m.f.: $C_9H_{10}O_2$, m.w.: 150	15.88	2928621	0.0768
Name: 3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate, m.f.: $C_{12}H_{20}O_2$, m.w.: 196	16.11	10476081	0.2747
Name: Phenol, 2-methoxy-3-(2-propenyl)-, m.f.: $C_{10}H_{12}O_2$, m.w.: 164	16.47	114481488	3.0019
Name: 9-Octadecene, (E)-, m.f.: $C_{18}H_{36}$, m.w.: 252	16.66	1044930	0.0274
Name: *n*-Decanoic acid, m.f.: $C_{10}H_{20}O_2$, m.w.: 172	16.74	2385572	0.0626
Name: Caryophyllene, m.f.: $C_{15}H_{24}$, m.w.: 204	17.48	5017894	0.1316
Name: Benzaldehyde, 3-hydroxy-4-methoxy-, m.f.: $C_8H_8O_3$, m.w.: 152	17.72	3311743	0.0868
Name: 2-Propenoic acid, 2-methyl-, 3-methyl-2-methylene-3-butenyl ester, m.f.: $C1_0H_{14}O_2$, m.w.: 166	18.00	1172008	0.0307
Name: Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, m.f.: $C_{15}H_{22}$, m.w.: 202	18.33	7655299	0.2007
α-Zingiberene, m.f.: $C_{15}H_{24}$, m.w.: 204	18.52	4386292	0.1150
Myristicin, m.f.: $C_{11}H_{12}O_3$, m.w.: 192	18.77	10321045	0.2706
Name: Phenol, 2,4-*bis*(1,1-dimethylethyl)-, m.f.: $C_{14}H_{22}O$, m.w.: 206	19.01	19329832	0.5069
Name: τ-Cadinol, m.f.: $C_{15}H_{26}O$, m.w.: 222	19.09	3925454	0.1029
Name: Elemol, m.f.: $C_{15}H_{26}O$, m.w.: 222	19.58	28615998	0.7504
Name: Diepi-α-cedrene epoxide, m.f.: $C_{15}H_{24}O$, m.w.: 220	20.15	890574	0.0234
Name: Caryophyllene oxide, m.f.: $C_{15}H_{24}O$, m.w.: 220	20.19	370887	0.0097
Name: α-Bisabolol, m.f.: $C_{15}H_{26}O$, m.w.: 222	20.49	7345865	0.1926
.Zingiberone, m.f.: $C_{11}H_{14}O_3$, m.w.: 194	21.26	17343860	0.4548
Name: Apiol, m.f.: $C_{12}H_{14}O_4$, m.w.: 222	21.51	21852672	0.5730
Name: 6-Octen-1-yn-3-ol, 3,7-dimethyl-, m.f.: $C_{10}H_{16}O$, m.w.: 152	21.64	5584531	0.1464
Name: 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-, m.f.: $C_{15}H_{24}O$, m.w.: 220	21.84	13838941	0.3629
Name: N-Isobutyl-(2E,4Z)-octadienamide, m.f.: $C_{12}H_{21}NO$, m.w.: 195	22.38	8447631	0.2215
Name: Tetradecanoic acid, m.f.: $C_{14}H_{28}O_2$, m.w.: 228	23.01	4389323	0.1151
Name: 3-Eicosene, (E)-, m.f.: $C_{20}H_{40}$, m.w.: 280	23.11	5826440	0.1528
Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, m.f.: $C_{20}H_{40}O$, m.w.: 296	24.06	2775829	0.0728
(+)-α-Bisabolol, m.f.: $C_{15}H_{26}O$, m.w.: 222	24.24	15606878	0.4092
α-Eudesmol, m.f.: $C_{15}H_{26}O$, m.w.: 222	24.79	8483221	0.2224
Cinnamic acid, methyl ester, (E)-, m.f.: $C_{10}H_{10}O_2$, m.w.: 162	26.73	607957120	15.9416
Name: 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0(2,8)]decan-7-one, m.f.: $C_{14}H_{22}O_2$, m.w.: 222	27.60	739981632	19.4035
Name: *n*-Hexadecanoic acid, m.f.: $C_{16}H_{32}O_2$, m.w.: 256	28.05	319181504	8.3695
Name: Phenol, 2-cyclohexyl-, m.f.: $C_{12}H_{16}O$, m.w.: 176	28.59	13904054	0.3646
.Nerolidol, m.f.: $C_{15}H_{26}O$, m.w.: 222	28.96	6318975	0.1657
Name: Naphthalene, decahydro-1,1-dimethyl-, m.f.: $C_{12}H_{22}$, m.w.: 166	29.91	14423179	0.3782
Piperic acid, m.f.: $C_{12}H_{10}O_4$, m.w.: 218	30.74	79137096	2.0751
Name: 2-Propenamide, N-octyl-3-phenyl-, m.f.: $C_{17}H_{25}NO$, m.w.: 259	31.22	92154048	2.4164
Name: Z,E-3,13-Octadecadien-1-ol, m.f.: $C_{18}H_{34}O$, m.w.: 266	31.96	410132608	10.7544
Name: Gingerol, m.f.: $C_{17}H_{26}O_4$, m.w.: 294	33.07	32617946	0.8553
Name: Gingerol, m.f.: $C_{17}H_{26}O_4$, m.w.: 294	34.42	252434752	6.6193
Name: 2-(3,4-Methylenedioxyphenyl)cyclohexanone, m.f.: $C_{13}H_{14}O_3$, m.w.: 218	34.82	280951136	7.3670
Name: 2-Propenamide, N-dodecyl-3-phenyl-, m.f.: $C_{21}H_{33}NO$, m.w.: 315	38.16	90135288	2.3635
Name: Lanost-8-en-3-ol, (3α)-, m.f.: $C_{30}H_{52}O$, m.w.: 428	39.26	55009816	1.4424
Name: Piperine, m.f.: $C_{17}H_{19}NO_3$, m.w.: 285	45.08	301235584	7.8989

other aromatic compounds from *Zingiber officinalis* and piperine from *Piper* species. These identified molecules are proven antioxidants and immuno-modulatory agents. It is inferred that this formulation must be working in HIV/AIDS patients probably through improving their antioxidant status and boosting their immune system.

ACKNOWLEDGEMENTS

The authors are thankful to Vice-Chancellor of SASTRA University, Thanjavur for utilizing the facilities to carry out the research work and Indian Council of Medical Research (ICMR), New Delhi for funding (No. 61/6-3/2007-HIV/DMS/TRM Dt. 29.05.08).

REFERENCES

1. Anonymous, The Siddha Formulary of India Part-I, edn 1, Ministry of Health and Family Welfare, New Delhi, p. 152 (1979).
2. B. Kaurinovic, S. Vlaisavljevic, M. Popovic, D. Vastag and M. Djurendic-Brenesel, *Molecules*, **15**, 5943 (2010).
3. S. Banerjee, H.I. Mullick and J. Banerjee, *Int. J. Pharm. Biol. Sci*., **2**, 283 (2011).
4. I. Elisia and D.D. Kitts, The Antioxidant Potential of Parsley and its Constituents; CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, *Nutrition and Natural Resources*, **3**, 1 (2009).
5. S. H. Kim, S. Y. Lee, C. Y. Hong, K. S. Gwak, M. J. Park, D. Smith, I. G. Choi, *Int. J. Cosmet. Sci*., **35**, 484 (2013).
6. F. Amezouar, W. Badri, M. Hsaine, N. Bourhim and H. Fougrach, *J. Appl. Pharm. Sci.*, **2**, 212 (2012).
7. Y. Masuda, H. Kikuzaki, M. Hisamoto and N. Nakatani, *Biofactors*, **21**, 293 (2004).
8. J. Upadhyay, R.K. Kesharwani and K. Misra, *Bioinformation*, **4**, 233 (2009).
9. R. Mittal and R.L. Gupta, *Methods Find Exp. Clin. Pharmacol*., **22**, 271 (2000).
10. S.K. Bhattacharya, K.S. Satyan, S. Ghosal, *Indian J. Exp. Biol*., **35**, 236 (1997).
11. S.K. Verma and A. Kumar, *Asian J. Pharm. Clin. Res.*, **4**, S1 (2011).
12. K.E. Heim, A.R. Tagliaferro and D.J. Bobilya, *J. Nutr. Biochem*., **13**, 572 (2002).
13. P. Akbay, A.A. Basaran, U. Undeger and N. Basaran, *Phytother. Res*., **17**, 34 (2003).
14. E.J. Middleton, *Adv. Exp. Med. Biol*., **439**, 175 (1998).