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LC-MS Analysis and Standardization of Khadirarishta – A polyherbal

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ABSTRACT:

A novel liquid chromatographic–Time of Flight mass spectrometric (LC-TOF-MS) method has been developed for the determination of Khadirarishta, polyherbal fermented Ayurvedic medicine made out by heart wood of Acacia catechu along with other herbal ingredients. It is having anti-lipid peroxidation activity with human erythrocytes. Results indicate that khadirarishta has good antioxidant potential and reduces lipidperoxidation in human erythrocytes. To determine the content, LC-MS study was carried out of herbal formulation.The chromatographic separation was achieved within 30 min by using Water (0.1% Formic acid): Acetonitrile as mobile phase with gradient system and Agilent Zorbax C18 column (100 x 2.1 mm, 1.8µm), the flow-rate was 0.3 ml/min. Ion signals m/z between 40 to 1700 was measured in the positive mode. The method was simple, sensitive, precise, accurate and was successfully applied to the herbal formulation and individual standard compound.

KEYWORDS: Khadirarishta, Lipid peroxidation, LC-MS, herbal formulation, Anti-oxidant.

INTRODUCTION:

Khadirarishta is one of the ancient polyherbal fermented liquid formulations prescribed in Ayurveda to cure all chronic disease, cardiac disorder, anemia, tumors, abdominal tumors, cysts, cough, intestinal worms and asthma, etc.[1] *Khadirarishta* is mainly made out of the *Acacia catechu* (heart wood) along with some other herbs and spice plants. It is known that oxidation of biological molecule like lipid leads to formation of H_2O_2 which ultimately leads to produces several disease conditions. Lipids are the most vulnerable to attacks of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the lipid peroxidation products can be used as potential biomarkers for indicating oxidative stress.[2]

Lipid peroxidation generates potentially toxic products which are chemically reactive and covalently modify critical macromolecules such as proteins [3], DNA bases [4][5] and low density lipoprotein (LDL) to proatherogenic forms. Lipid peroxidation has also been implicated in the neuro degeneration.[6] The increased level MDA (malondialdehyde) is known to cause hemoglobin denaturation, membrane lipid peroxidation, cross-linking between membrane skeletal proteins and between membrane and hemoglobin.

The levels of MDA and TBRS (thiobarbituric acid reactive substance) reacting substances (lipid peroxidation products) have been measured extensively to detect oxidative stress levels in biological fluids and human erythrocytes.[7]

The in vitro study was done to determine MDA level in erythrocytes using *Khadirarishta which* shows presence of multi-antioxidant compounds which might have synergistically contributed to restrain lipid peroxidation of human erythrocytes. [8] The content of *Khadirarishta* sample must be identify to support the results of invitro study and for presence of anti-oxidant compounds. So LC-MS method is the best way to find out the different compound present in given herbal formulation.

LC-MS is a powerful technique used for many applications which has very high sensitivity and

specificity. Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

It provide information about molecular weight, structure, identity and quantity of the substance in specific sample components.

EXPERIMENTAL

Materials

Khadirarishta sample was procured from Ayurvedic Rasashala, Karve Road, Pune. Acetonitrile (HPLC grade) was obtained from Merck, Darmstadt, Germany. Formic acid was supplied by E. Merck (India) Ltd., Mumbai, India. HPLC grade Water from Millipore's Milli-Q System was used throughout the analysis.6540 ultra-high definition accurate mass QTOF LC/MS system was used. All MS acquisitions were performed in the positive electrospray ionization mode. The capillary voltage, cone voltage, fragmentor voltage were 4 kV, 45V and 170V, respectively. The gas temperature was set at 325 deg. Data was acquired at scan rate of 3Hz in mass range 40-1700 m/z. Further data was analyzed with Mass hunter qualitative software and METLIN database.

Chromatographic and MS conditions

Chromatography was performed on LC–MS system from Agilent 1260 binary LC System. The data acquisition was carried out on Mass hunter qualitative software and METLIN database. Chromatographic separation was achieved on Agilent Zorbax C18 column (100x2.1 mm, 1.8µm) analytical column. The mobile phase consisting of Solution A: Water (0.1% Formic acid) and Solution B: Acetonitrile was delivered at a flow rate of 0.3 ml/min with different proportion (Table 1). 1 µL sample was injected into LC–MS. The gas temperature was 325º C. The nitrogen gas flow was maintained at 10 l/min and for nebulization20 l/min. Single ion monitoring (SIM) of the ions was carried in positive mode and the ion signals were obtained in range of 40-1700 m/z ratio. The Sheath Gas Temp was 320º C and Sheath Gas flow was 10 l/min. The Scan rate was 3 spectra/sec. A representative interpretation of mass spectrais exhibited in table 2

Table: 1 Gradient proportion of Mobile Phase of LC

Sr. No.	Time	% A	%B
1	5.00 min	95	5
2	15.00 min	60	40
3	25.00 min	15	85
4	28.00 min	5	95
5	30.00 min	95	5

RESULTS AND DISCUSSION

The MS spectra interpretation is shown in Table 2.

Table: 2 Interpretation of MS spectra

Sr. no.	Compound Label	RT	Mass	Name	Formula
1	Cpd 6: SNAP (S-NITROSO-N-ACETYLPENICILLAMINE)	0.68	220.0516	SNAP (S-NITROSO-N-ACETYLPENICILLAMINE)	C7 H12 N2 O4 S
2	Cpd 13: Epimelibiose	0.77	342.1169	Epimelibiose	C12 H22 O11
3	Cpd 14: Glycerophosphocholine	0.77	258.1099	Glycerophosphocholine	C8 H21 N O6 P
4	Cpd 15: Ethosuximide M5	0.82	155.0586	Ethosuximide M5	C7 H9 N O3
5	Cpd 16: 3-Hydroxynorvaline	0.83	133.0736	3-Hydroxynorvaline	C5 H11 N O3
6	Cpd 17: Maltotriose	0.85	504.1696	Maltotriose	C18 H32 O16
7	Cpd 18: Queuine	0.85	277.1167	Queuine	C12 H15 N5 O3
8	Cpd 19: Epimelibiose	0.86	342.1166	Epimelibiose	C12 H22 O11
9	Cpd 20: L-Carnitine	0.86	162.1133	L-Carnitine	C7 H16 N O3
10	Cpd 22: Betaine aldehyde	0.9	102.0924	Betaine aldehyde	C5 H12 N O
11	Cpd 25: Deoxythymidine monophosphate (dTMP)	0.91	322.0565	Deoxythymidine monophosphate (dTMP)	C10 H15 N2 O8 P
12	Cpd 27: Gallic acid	0.91	170.0224	Gallic acid	C7 H6 O5
13	Cpd 30: nicotinamide mononucleotide	1.08	334.0553	nicotinamide mononucleotide	C11 H15 N2 O8 P
14	Cpd 31: DIPYROCETYL	1.08	238.047	DIPYROCETYL	C11 H10 O6
15	Cpd 32: ,4-Dihydroxyphenylpropionic acid	1.09	182.0575	,4-Dihydroxyphenylpropionic acid	C9 H10 O4
16	Cpd 34: D-Glutamate	1.1	147.0532	D-Glutamate	C5 H9 N O4
17	Cpd 35: 1-Aminocyclopropane-1-	1.1	101.0478	1-Aminocyclopropane-1-	C4 H7 N O2

	carboxylic acid			carboxylic acid	
18	Cpd 37: Salicyl acyl glucuronide	1.28	314.064	Salicyl acyl glucuronide	C13 H14 O9
19	Cpd 39: Mezlocillin metabolite (4-Thiazolidinecarboxylic acid, 5,5-dimethyl-2-[[[[[[3-(methylsulfonyl)-2-oxo	1.34	513.1354	Mezlocillin metabolite (4-Thiazolidinecarboxylic acid, 5,5-dimethyl-2-[[[[[[3-(methylsulfonyl)-2-oxo	C20 H27 N5 O7 S2
20	Cpd 42: Gallic acid	1.5	170.0211	Gallic acid	C7 H6 O5

From the LC-MS result, it is concluded that khadirarishta sample contains 20 different types of constituent in it including anti-oxidant compounds like Gallic acid. Furthermore it also contains flavonoids, phenolic compounds and phenol derivatives which include Epimelibiose, Maltotriose, Queuine, Deoxythymidine monophosphate (dTMP), Gallic acid, Nicotinamide mononucleotide etc.

It has been accepted that flavonoids act as antioxidants through scavenging or chelating process andplay significant role in human health and fitness [9]

Phenolic and flavonoids compounds have health applications as they are recognized as potent antioxidants, exerting antioxidative function asterminators of free radicals and chelating metals that are capable of catalyzing lipid peroxidation. They may act by donating a hydrogen atom to radicals, which results in the formation relatively stable phenoxy radical intermediates, making it more difficult for a new chain reaction to initiate. [10]

The antilipid peroxidation capacity of *khadirarishta* can be attributed to presence of phenols and flavonoids including gallic acid in it. This activity might be due to existence of multiple hydroxyl groups in each phenolic compound which might have donated their protons to break the chain reaction of free radicals [11] and inhibited lipid peroxidation of erythrocyte membranes. In present study we conclude that *khadirarishta* showed presence of multi-antioxidant compounds which might have synergistically contributed to restrain lipid peroxidation of human erythrocytes.

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