IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
October 2020 Vol.:16, Issue:4

Ferulic Acid and Maleamic Acid - The Probable Scientific Basis of Pairing of *Meda-Mahameda* (*Polygonatum cirrhifolium* and *P. verticillatum*) Couplets

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Submission: 20 September 2020
Accepted: 26 September 2020
Published: 30 October 2020

Keywords: Anti-aging, Anti-oxidant, Ferulic acid, Maleamic acid, *Meda-Mahameda*.

ABSTRACT

Ashtawarga is a group of eight rare plants used in a plethora of aphrodisiacs *Ayurvedic* formulations and has been divided into four pairs known as *Meda-Mahameda, Kakoli-Kshirakakoli, Jeevaka-Rishbhaka,* and *Riddhi-Vriddhi*. The Department of AYUSH has recommended the use of substitutes in the absence of authentic plants; however, the use of substitutes may spoil the clinical/therapeutic efficacy of concerned formulations. Probably our ancient scholars were aware of the scientific reasons for the pairing of these plants in that era; however, no ancient text has clearly mentioned the basis of the pairing of these plants. Hence, it may be of great importance to find out the scientific logic behind this pairing. The pairing of plants either may complement each other in therapeutic action or may exert some additive/synergistic effect. The authors highlight the basis of this pairing on the basis of common chemical markers. In our previous studies, anti-oxidant markers ferulic acid and maleamic acid have been isolated from *Mahameda* and *Meda* (respectively). The parallel Thin Layer Chromatography (TLC) of selected markers with *Meda* and *Mahameda* was done to detect the presence of markers. *Mahameda* was shown the presence of both markers ferulic acid and maleamic acid. Ferulic acid is a potent antioxidant compound that may have an additive or synergistic effect on the anti-oxidant, aphrodisiac, and anti-aging activity of maleamic acid present in both the plants. The presence of active component maleamic acid in both plants *Meda* and *Mahameda* seems to be the basis of the pairing of these plants.

INTRODUCTION

Ashtawarga is a group of eight plants viz. *Meda* (*Polygonatum cirrhifolium* (Wall.) Royle), *Mahameda* (*P. verticillatum* (L.) All.), *Kakoli* (*Roscoea procera* Wall. synonym *R. purpurea*), *Kshirakakoli* (*Fritillaria roylei* Hook. f), *Jeevaka* (*Microstylis muscifera* Ridley), *Rishbhaka* (*Malaxis acuminata* D. Don), *Riddhi* (*Habenaria edgeworthii* H. f.) and *Vriddhi* (*H. intermedia* D. Don) [1]. *Ashtawarga* plants are being widely used in a plethora of *Ayurvedic* formulations specifically used as aphrodisiacs and rejuvenators [2,3]. These are considered under threatened conservation status due to limited distribution; however, the market demand of these plants is increasing day-by-day [3,4].

Keeping in view the unavailability of these raw drugs in the market, the Department of AYUSH has permitted the substitution of these plants with other easily available plants known as substitutes (*Pratinidhi dravyas*), regarded as the official substitutes [2,5,6]. Ancient Vedas like Bhavamishra (16th Century) have also suggested the substitutes of *Ashtawarga* plants in his book Bhavaprakasha Nighantu and have divided the original eight plants into four pairs i.e. *Meda-Mahameda, Kakoli-Kshirakakoli, Jeevaka-Rishbhaka,* and *Riddhi-Vriddhi* [7]. Even the plants in the pairs have been suggested to be used as substitutes for each other e.g. *Meda* can be substituted with *Mahameda* and vice versa [8]. Similar is the case of plants in other pairs of *Ashtawarga* plants. The pairing of plants must be having some valid scientific basis but it has not been mentioned in the ancient texts. Our ancient scholars must be aware of the scientific reasons for the pairing of these plants in that era; however, no text has mentioned for the basis of the pairing of these plants [9,10]. It is quite possible that *Ashtawarga* plants might be complementing or supplementing some therapeutic actions for each other due to the phytoconstituents. Hence it may be of great importance to find out the scientific logic behind this pairing. One of the important assumptions could be the common active component that could have an additive effect in the formulation whereas other reasons could be an active component that is complementary in action to the active component of other plants. In previous studies, an effort was made to isolate marker compounds of *Meda* and *Mahameda* pair to establish the basis of this pairing in these plants on the basis of chemical markers [11-13].

MATERIALS AND METHODS

Chemicals, reagents, and instruments

All solvents and reagents used, were of analytical grade, procured from Qualikems, Finar, and Merck, etc. Silica gel (60-120mesh size / 0.120-0.250mm particle size) was used for column chromatography. Silica gel 60 F_{254} pre-coated aluminum sheets were used for Thin Layer Chromatography (TLC). Different spectroscopic techniques such as Infra-Red (IR), Nuclear Magnetic Resonance (NMR), and Mass spectra were used to identify the structure of the isolated markers. An IR spectrum was recorded on FTIR Perkin Elmer, NMR spectrum was recorded on Bruker Advance II 400 NMR spectrometer and Mass spectrum was recorded on Mass Spectrometer Model Q-ToF Micro Waters equipped with Electrospray Ionization (ESI) at Panjab University, Chandigarh.

Plant material

Rhizomes of *Meda* and *Mahameda* were procured from an approved cultivator of Himalayan Research Group (HRG) having field station at Village Dhangiara, Distt Mandi (Himachal Pradesh). The plant samples of *Meda* and *Mahameda* were authenticated by Central Instrumentation Facility (National Botanical Research Institute, Lucknow) and by HRG vide reference numbers NBRI/CIF/524/2016 and HRG/Testimonial-NMPB/02/2015-2016 [11-12]. Crude plant samples were dried under shade (<40°C). The dried material of each plant was coarsely powdered and stored in a desiccator for future use.

Extraction and isolation of markers

In the previous publications, the coarsely powdered rhizomes of *Meda* and *Mahameda* were extracted with methanol through a continuous hot maceration process. The extracts were filtered and the filtrates were concentrated by distillation to obtain a semi-solid residue. Methanolic extracts of *Meda* and *Mahameda* were subjected to column chromatography. The number of fractions was collected with an optimum flow rate of 4 ml/min and the mobile phase for TLC of fractions was standardized by hit and trial method by using the solvents of different polarities. The fractions with similar TLC profiles were pooled to give major fractions. The single compounds were seen on TLC plate under UV-Visible spectrophotometer at different absorption

spectrum (λ_{max}) and these compounds were cut with sharp-edged scissors for the isolation purpose. The isolated markers were purified by crystallization with methanol and characterized as ferulic acid and maleamic acid [11-12].

Comparative TLC

Another experiment TLC was designed to know the common component of both the plants. The isolated markers (Ferulic acid and maleamic acid) were used as reference markers and run against plant extracts of *Meda* and *Mahameda*. Standard TLC plates were activated into hot air oven at 105°C for 10min. The mobile phase was optimized in the ratio n-hexane: ethyl acetate: formic acid (4:6:0.1v/v/v) and allowed to saturate for 15min. The plant extracts and isolated markers were spotted on the plate and TLC plate was placed into the TLC chamber. As the solvent reached near the top of the TLC plate, the plate was removed, dried, and visualized using UV light of UV-Visible spectrophotometer. The presence of the markers was detected at λ_{max} (254nm and 365nm). Parallel TLC of *Meda*, *Mahameda*, and their isolated markers was observed.

RESULTS

Spectral analysis of selected markers

In the previous studies, the isolated markers have been identified as ferulic acid from *Mahameda* and maleamic acid from *Meda* through IR, NMR, and Mass spectral analysis (Table 1, Figure 1-6). The figures (Figure 1-6) were adopted from Virk et al. 2016, 2017 [11-12].

Table No. 1: Spectral analysis of selected markers.

Spectra	Selected markers	
	Ferulic acid [11]	**Maleamic acid** [12]
IR spectrum	3436cm^{-1} (phenolic O-H stretching), 1690cm^{-1} (carbonyl C=O stretching), 1273cm^{-1} (carboxylic acid C-O stretching), 1514 and 1690 cm^{-1} (aromatic C=C) (Figure 1).	2921.62cm^{-1} for C=C-H (*Cis*-olefins, stretching), 2852.8cm^{-1} for NH (carboxamide), 1732.94cm^{-1} for C=O (carboxylic acid), 1459.88cm^{-1} for C-O and C-N stretching, 1377.93cm^{-1} C=C stretching, 1275.94cm^{-1} for -C-O stretching, 1073.89cm^{-1} for *cis*-NH_2 and COOH interaction, 722.96cm^{-1} (*cis*-C=C- bending) (Figure 2).
NMR spectrum	δ: 3.845 (3H, s, H-4'), 6.29 (1H, d, J=15 Hz, H-2'), 6.79 (1H, d, J=8 Hz, H-6), 7.2 (1H, d, J=8 and 2 Hz, H-5), 7.47 (1H, d, J=2 Hz, H-3), 7.02 (1H, dd, J=8 Hz, H-1') (Figure 3)	δ: 9.54 (s, 1H, O-H), 8.53 (s, 2H, NH_2), 7.44-7.45 (d, 1H, *J* = 3.52 Hz, CH), 6.57-6.6.58 (d, 1H, *J* = 3.52 Hz, CH) (Figure 4)
Mass spectrum	M/z 427 (Dimer + K^+), fragmentation peaks at 177 (M-OH), 145 (M -OH-OCH_3) and 621 (3M + K) (Figure 5)	Fragmentation peak at 97 (M-OH), 116 (M+1) and 231 (2M+1) (Figure 6)
IUPAC name	4-hydroxy-3-methoxy-cinnamic acid	(*Z*)-4-amino-4-oxobut-2-enoic acid
Isolated from	*Mahameda*	*Meda*

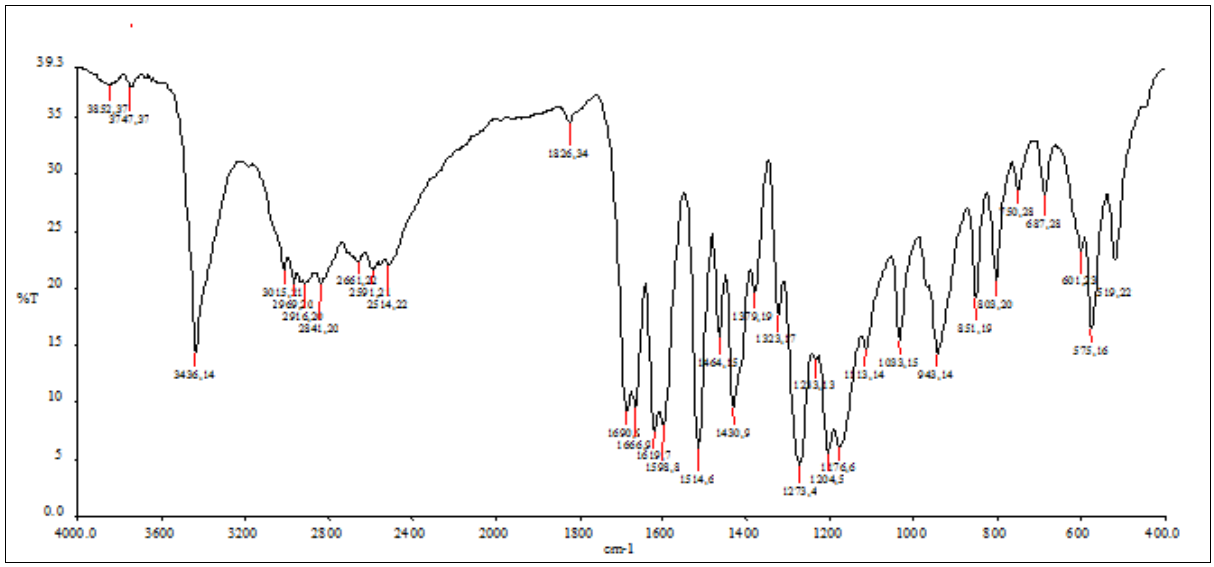

Figure No. 1: IR spectrum of ferulic acid.

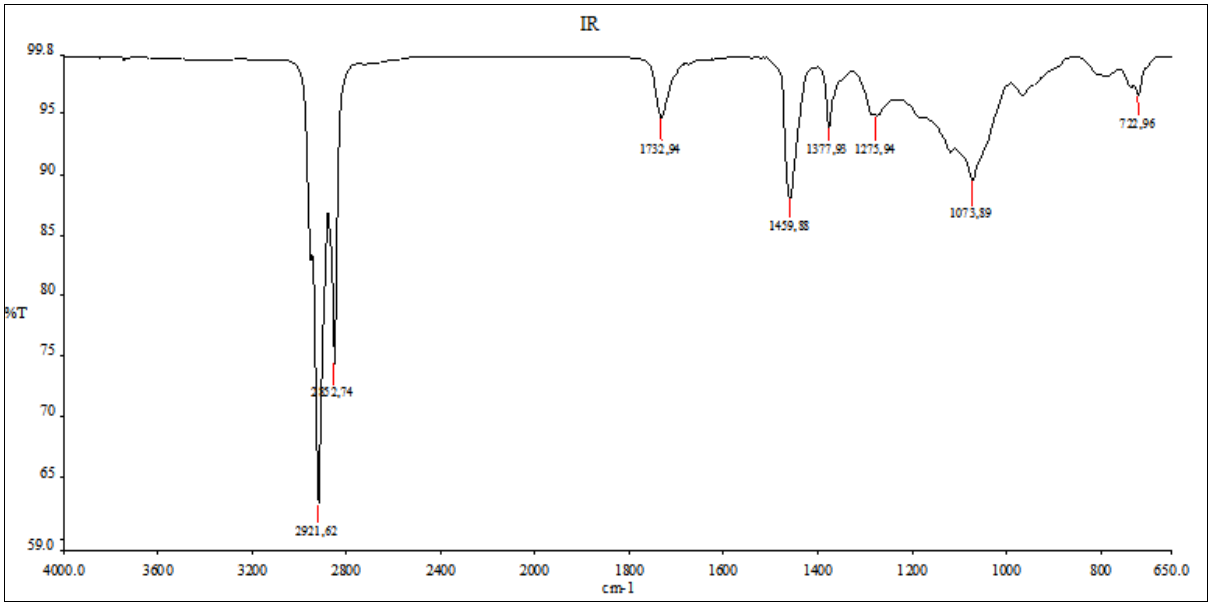

Figure No. 2: IR spectrum of maleamic acid.

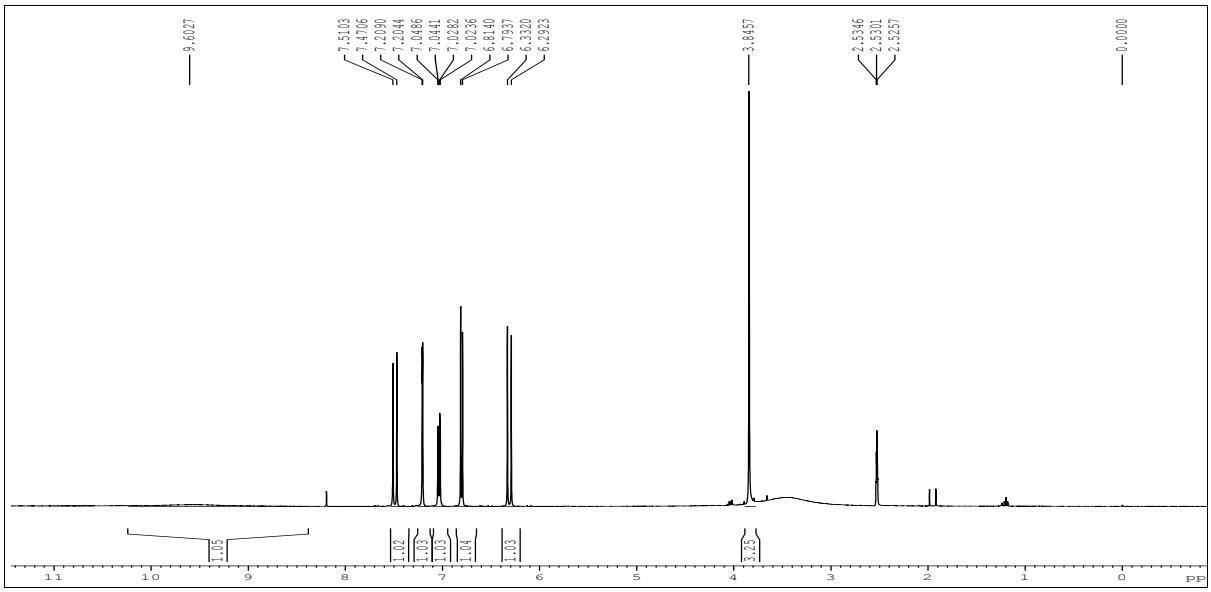

Figure No. 3: NMR spectrum of ferulic acid.

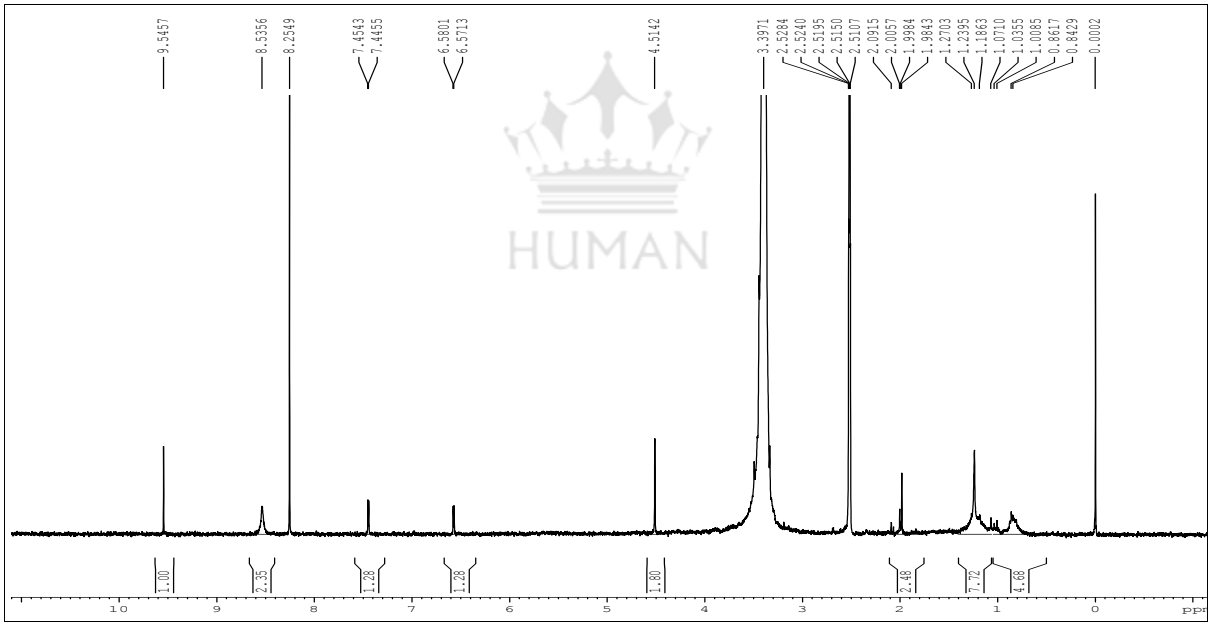

Figure No. 4: NMR spectrum of maleamic acid.

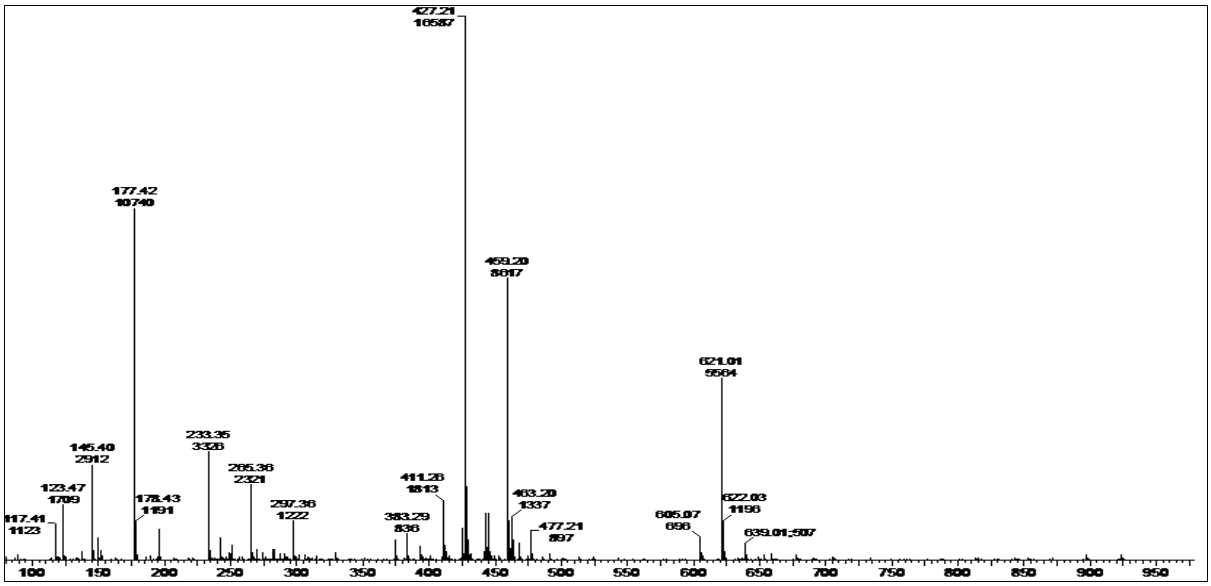

Figure No. 5: Mass spectrum of ferulic acid.

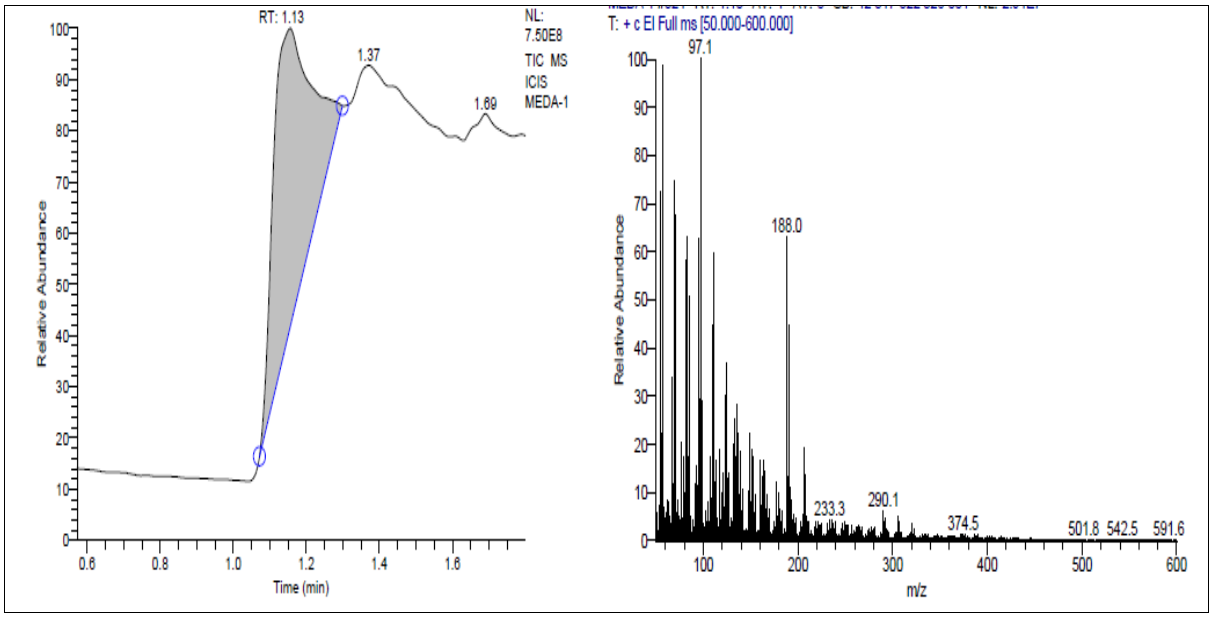

Figure No. 6: GC-MS of maleamic acid.

Comparative TLC

In TLC, the best separation of ferulic acid and maleamic acid was observed at λ_{max} 254nm. R_f values of all the spots were measured from TLC plate (Figure 7). R_f values of standard ferulic acid and maleamic acid were found to be 0.83 and 0.79, respectively (Table 2). The corresponding spots to markers ferulic acid and maleamic acid were found in *Mahameda*

whereas only one spot corresponding to maleamic acid was found in *Meda*. The results clearly indicate the presence of maleamic acid in both the plants whereas ferulic acid is an extra marker found in *Mahameda*.

Table No. 2: R_f values**.**

***Ashtawarga* pair**	**R_f of selected markers**	
	Ferulic acid (R_f)	**Maleamic acid (R_f)**
Meda	Absent	Present (0.79)
Mahameda	Present (0.83)	Present (0.79)

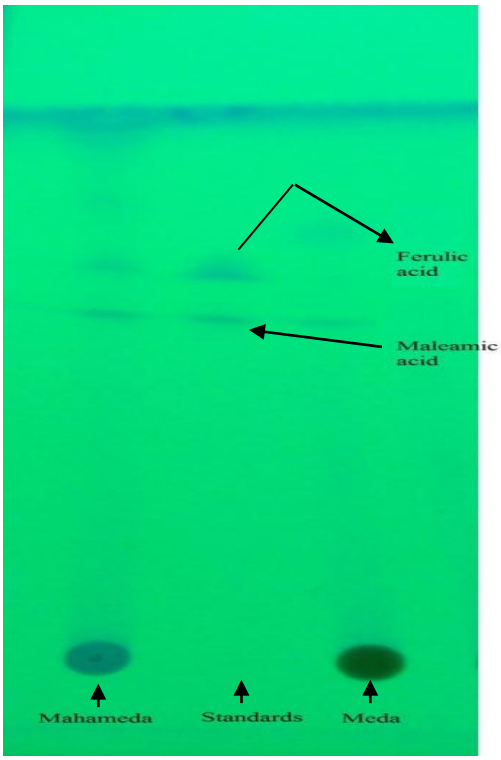

Figure No. 7: TLC of *Meda-Mahameda* pair and the selected markers**.**

DISCUSSION

The theory of substitute has been given by Bhavmishra (sixteenth Century A.D.). The number of substituted plants in *Ayurveda* has expanded enormously while the genuine idea of substitute has

been disregarded [14]. *Ayurveda* suggests that practically comparable substitutes having comparative *Guna-Karma* characteristics can be utilized in the nonexistence of original medicinal species. It likewise expresses that *Rasa* (taste) of an herb relies upon its pharmacological action (*Karma*) [15]. Acharya Vagbhata, Acharya Bhavamishra, and Yogaratnakara expressed that if *Rasa, Guna, Virya,* and *Vipaka* of one drug are like another, at that point it qualifies a plant to be chosen as a substitute [16-19]. Bhaishajya Ratnavali has announced that the chief drug of the formulation cannot be subbed but only the frill drugs of the formulation can be supplanted by appropriate *Pratinidhi dravyas* [20]. A literature review discloses that over 33% *Ayurvedic* parameters of *Rasapanchakas* as well as pharmacological actions of *Meda* and *Mahameda* don't coordinate with each other. Simultaneously, *Guna* and pharmacological actions of *Mahameda* and *Meda* are not exactly similar (Table 3).

Table No. 3: Comparison of *Rasapanchakas* and pharmacological actions of *Meda-Mahameda*.

***Ashtawarga* pair**	**Similarity in**					
	Rasa	***Guna***	***Virya***	***Vipaka***	***Doshic action***	**Pharmacological action**
Meda and *Mahameda*	√	×	√	√	√	×

All *Ayurvedic* characteristics are correlated to each other. If even a solitary parameter is changed, the remedial action of the drug may likewise alter. This shows that *Ayurvedic* rationale for pairing doesn't fit to the circumstance and is disregarded [21].

World Health Organization has mentioned the dismissal of crude material, having over 5% of some other plant part of the similar plant despite the fact that it might be gotten from the authentic plant. According to these norms, adulterated drugs (purposeful or inadvertent) should be dismissed [22]. As per GMP rules & act in schedule-T for *Ayurvedic, Siddha,* and *Unani* (ASU) drugs in segment 33EEA, ASU drugs are regarded to be unauthentic if it is a substitute for another drug or on the off chance that it has been substituted by other drug [23]. To circumvent these problems, most monographs define maximum permissible limits of the foreign matter often based on the TLC test using chemical markers allowing a distinction between the

correct species and other potentially toxic species [23-25]. Hence, in the present study, TLC method has been employed to define the presence of chemical markers in these plants.

The presence of maleamic acid in both plants representing *Meda-Mahameda* pair indicates a strong correlation for the pairing of these two plants. It is pertinent to mention here that maleamic acid is a very strong anti-oxidant and could be indicative of the previously published role of both the plants in anti-aging action of preparation/formulations containing these plants [2,26]. It is very important to mention here that ferulic acid is also an established anti-oxidant [27]. So here the probability of supplementation and complementation of anti-aging effect of both the plants cannot be ignored. *Meda* and *Mahameda* pair may have been constituted keeping in view the anti-oxidant activity of both these plants containing maleamic acid. The nomenclature of *"Maha" in Mahameda* in comparison to *Meda* could have been designed on the basis of two components of the plant with anti-oxidant activity (Ferulic acid + maleamic acid) present in *Mahameda* whereas single maleamic acid is present in *Meda*.

CONCLUSION

Meda-Mahameda plants have been recommended in *Ayurveda* as *Jeevaniya* drugs that are meant for rejuvenation of the body and this fact seems to be directly correlated with the anti-oxidant activity of the plants. In Hindi, the word "*Maha*" is used to demonstrate better strength of an object/subject. It was found that *Meda* and *Mahameda* had a common marker known as maleamic acid, whereas *Mahameda* has an additional marker ferulic acid that may have an additive or synergistic effect on the aphrodisiac and anti-aging activity of maleamic acid present in both the plants. From this study, it may be concluded that the presence of the same marker is probably the scientific reason for pairing *Meda-Mahameda* pair. Further studies are needed to establish such more scientific evidence for the basis of the pairing of these important medicinal plants.

ACKNOWLEDGMENT

The authors express sincere thanks to National Medicinal Plants Board, Department of AYUSH, Ministry of AYUSH, Govt. of India, New Delhi [Grant number: R&D/CH-01/2012] for providing the financial support to carry out this project.

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