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File name: IC_Allium_sativum_L_IN_BOILED_MUSTARD_Brassica_nigra_L....
File size: 3.15M
Page count: 92
Word count: 26,422
Character count: 139,009
Submission date: 12-Dec-2024 03:18PM (UTC+0530)
Submission ID: 2550077827

ABSTRACT

Secondary metabolites present in the garlic mustard oil macerate (GMM) may be responsible for the bioactivities claimed in traditional practices. Therefore, the determination of unique compounds in GMM is very important. Traditionally people crush garlic and prepare the macerate (GMM) in boiled (160°C) mustard oil but major compounds reported earlier are synthesized by crushing garlic in other vegetable oil and are found highest at 80°C. Therefore, GMM at 80°C and 160°C are prepared (GMM80 & GMM160). Three compounds namely, allyl isothiocyanate (AITC), 1-Butene, 4-isothiocyanato- (BITC), 2-vinyl-4H-1,3-dithiin (VD) (excluding the fatty acids) are found to be highly abundant after GCMS analysis of in GMM80. AITC and BITC are the compounds which are reported by earlier research groups to be present in MO. One of these compounds i.e., 2-Vinyl-4H-1,3-dithiin is unique to GMM80 and not found in MO and GMM160. A similar trend was found for antibacterial and antifungal activity where GMM80 is better in many cases. Therefore, it is concluded that GMM80 is better and all further experiments will be carried out with GMM80. Compounds present in GMM80 are also identified by liquid chromatography-mass spectrometry (LC/MS). Two major compounds that were identified are ajoene (AJ) and 2-Vinyl-4H-1,3-dithiin (VD) (which was also detected during GCMS analysis). Since literature suggests AJ and DE production depends on oil quality, temperature, time of heating, and garlic to MO ratio, therefore, optimization was carried out with respect to maximum AJ and DE synthesis by feeding different experimental data to response surface methodology (RSM) software. The preparation conditions for optimum production of AJ (garlic and mustard oil ratio 1:2, 55°C, 4.5 h) and VD (garlic and mustard oil ratio 1:2.22, 77.51°C, 2.5 h) were standardized. The optimum condition suggested by RSM was validated and used in all future experiments. The concentration of ajoene and vinyl dithiin in optimized GMM was found to increase by 1.75 and 1.83 folds then compared to the initially prepared GMM. This experiment optimized the traditional preparations with respect to two secondary compounds (AJ & DE) production.

Traditionally the GMM oil is applied on the nasal opening with the hope that the VOC will reach the nasal cavity and sinus and prevent cold-related secondary infections caused by bacteria and fungus. Therefore, GCMS and LCMS were carried out for the identification of the possible VOCs in GMM. To establish the hypothesis, VOCs in optimized GMM were tested against bacteria and fungus. Against *S. aureus*, GMM VOC was found to be decreasing staphyloxanthin (SX) which is a potent antibiotic resistance imparting molecule by 22.65%

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File name: IC_Allium_sativum_L_IN_BOILED_MUSTARD_Brassica_nigra_L._OIL.docx (3.15M)

Word count: 26422

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