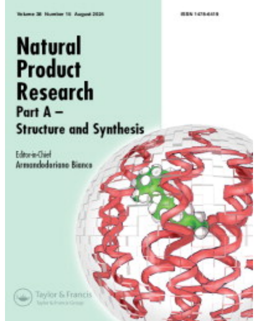

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Insights from degradation studies of alpha mangostin from *Garcinia mangostana*: key findings

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ABSTRACT

The present study emphasises the necessity of substantiating the stability of plant-derived bioactive compounds for their therapeutic effectiveness in pharmaceutical production. The limelight is on alpha-mangostin (AM), a xanthone from *Garcinia mangostana* L., renowned for its diverse biological properties. Acid exposure during a forced degradation study on AM resulted in degraded alpha-mangostin (DAM) formation, with structural modifications of the two prenyl groups at C2 and C8 positions as determined by NMR and HRMS analysis. Other conditions (temperature, humidity, photolytic, oxidative, and alkaline) showed a minimal impact on AM. DAM, although showed antibacterial activity at concentration higher than AM (MIC values for AM: 0.39–1.56 µg/mL; DAM: >25 µg/mL), it exhibited potential for binding with Glucosyltransferase-SI from *Streptococcus mutans* and human Acetylcholinesterase in molecular docking simulations, comparable to AM. This suggests, the importance of prenyl group at C2 and C8 positions for AM's potent antibacterial activity and the decreased activity of DAM is due to lack of the prenyl groups.

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1. Introduction

Xanthone derivatives, predominant in *Garcinia mangostana* include over 60 variants isolated from its various plant parts (Obolskiy et al. 2009; Sriyatep et al. 2015). Alpha-mangostin (AM) a major xanthone, comprising 78% of the content holds significant pharmacological value and popularly used in herbal cosmetics and drug preparations. (Ibrahim et al. 2016; Richard et al. 2017; Phumlek et al. 2022). With the emerging cases of antibiotic resistance in bacteria, the need of the hour is to use natural plant-based bio-actives like AM, as resistance to such natural products has not yet been reported.

Stability assessments are pivotal in evaluating product shelf life, storage conditions, and quality for consumer use (Khan et al. 2010; Bajaj et al. 2012). These tests monitor changes in physical, chemical, biological, and microbiological attributes of pharmaceuticals. International Conference on Harmonisation (ICH) guidelines, such as "Stability Testing of New Drug Substances and Products" (Q1A), outline methods to identify degradation products and pathways. Stress testing, as conducted in this study, investigates the impact of test parameters on the stability of active ingredients like AM, aligning with recommendations by Singh and Bakshi.

The current study was therefore, focused to determine whether the forced degradation affects the structure of AM and if such a degraded compound maintains biological activity. Identification of the structural changes that the compound AM undergoes was observed through TLC, HPTLC, HPLC, NMR and HRMS studies. Biological activity was determined in terms of its antimicrobial properties when tested against bacterial pathogens like *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium*, *Streptococcus pyogenes* and *Propionibacterium acnes*. To further determine the effect on biological activity of the compound AM and degraded compound, molecular docking simulations were employed against two target proteins: Glucosyltransferase-SI from *Streptococcus mutans* and human Acetylcholinesterase based on the literature demonstrating AM's activity against these target proteins.

2. Results and discussion

2.1. Forced degradation studies

Forced degradation of AM was conducted as a part of stability assessment to understand its breakdown pathways, elucidate degradation product structures, and evaluate inherent stability. The forced degradation of AM showed that it is fairly stable at different thermal, alkali, photolytic, humidity, and oxidative conditions (Table S1), except at acidic conditions. Slight degradation of AM up to 6.9% was observed during the humidity degradation study when exposed to 40 °C/75% RH for 7 days, and up to 4.7% was observed when exposed to UV light of 1.2 million lux/h at 200 Watt-h/m sq. Since these values did not impact its structure and activity, the changes were not regarded as significant. A noticeable degradation was observed, which increased with higher acidic condition, starting from 14.1% at 0.1 N HCl for 1 h to 78.4% at 2 N HCl for 1 h. AM gets degraded and undergoes structural modification to form a different compound at 3 N HCl at 80 °C for 8 h.

2.2. TLC and HPTLC analysis

TLC and HPTLC were performed preliminary to understand the difference between AM and DAM. For TLC, the R_f values for AM and DAM were 0.62 and 0.36, respectively, when read at 254 nm under UV light (Figure S1, panel A) substantiating the fact that AM and DAM are two separate compounds. For further validation, HPTLC was performed, where 2 separate spots were observed with different Rf values: AM (Rf = 0.63) and DAM (Rf = 0.49) as illustrated in Figure S1, panel B.

2.3. HPLC analysis

The HPLC chromatograms of AM and DAM showed that both have a different retention time of 23.863 and 12.883 min, respectively when recorded at 243nm as described previously by Walker 2007. Thus, indicating the fact that AM and DAM are two different compounds. The purity of AM was found to be 98% pure (Figure S2). (UV Spectra for AM and DAM are slightly different as seen during HPLC analysis, Figure S3A and S3B respectively). DAM is presumed to have structural alteration, which is further elucidated by NMR and mass spectroscopy.

2.4. Antibacterial activity of AM and DAM

The MIC of AM against a panel of bacteria was found to be between 0.39 and 1.56 µg/mL (Table S2), whereas DAM did not show any inhibition in the same range. The structural alteration of AM due to acid treatment reduced its potency as an antimicrobial agent with MIC values >25 µg/mL. The MIC values of standard drug tetracycline used as positive control for the panel of bacteria used ranged between 0.5-160 µg/mL as reported in the literature (Sedigheh et al. 2010, Rachid et al. 2000, Huys et al. 2004, Nakase et al. 2017, Jasir et al. 2000). Prenylated xanthones from mangostin are known to have antimicrobial activity (Genovese et al. 2016). Thus, AM being a prenylated xanthone was found to be a potent antimicrobial compound, and structural modification under acidic conditions may be responsible for the increase in MIC. The results elucidate that DAM may not have lost its activity completely but have lost its potency as compared to AM and inhibits bacteria at a higher concentration range as compared to AM.

2.5. Characterisation of DAM

2.5.1. Physicochemical properties of DAM

AM was isolated and purified as mentioned earlier by Chaves et al. 2020 and subjected to forced degradation which upon acid treatment showed structural modification leading to the formation of DAM. DAM was pale yellow in appearance, and it was found to be soluble in DMSO, methanol, and ethanol. Melting point of AM was in the range of 183–185 °C with a mean melting point of 184 °C whereas DAM had an increased melting point in the range of 202–204 °C with a mean melting point of 203 °C.

2.5.2. NMR and mass spectroscopy analysis

The elemental analysis revealed the elements as C, 67.28; H, 6.59 and O, 26.14. The M + H value of 429.1838 was obtained by performing the mass spectrometry (Table

S3; Figure S4). The mass fragmentation pattern of the positive scan and negative scans can be seen in Figure S4 and the probable fragmentation pattern of the structure is given in Table S3. The NMR spectra in DMSO showed 28 protons after integration. Single proton of hydroxyl group were seen around 14.7, two aromatic protons were observed around the value 6.1 to 6.3, two protons of hydroxyl group around 4.1, three protons of methoxy group were observed at 3.7, eight protons of four CH_2 protons were observed in between 1.5-3.2 which confirms the modification of both prenyl groups at C2 & C8 positions, twelve protons of four methyl groups were observed around 1.2-1.3. In 13 C NMR, C-9 carbon has highest chemical shift value around 180 ppm due to carbonyl group; C-3 & C-6 carbon had values around 160 ppm due to the presence of a hydroxyl group; carbon numbers C-2, C-4, C-5, C-7, C-8 at chemical shift values around 90-150 ppm; and carbon numbers C-8a and C-9a showed values around100 -110 ppm (Figures S5–S7). Based on the LC-MS and NMR analysis, both the prenyl groups at C2 and C8 positions in AM gets modified, one gets oxidised and the other one cyclised during acid degradation. Prenylated compounds are generally susceptible to oxidative degradation, and cyclisation particularly in the presence of catalytic mediators and also under physiological conditions (Tang et al. 2021). AM when treated with OsO_4 and NMO is reported to generate three derivatives wherein the prenyl groups of C2, C8 and both positions get oxidised with yields of 10%, 12% and 78% respectively (Chi et al. 2018). Thus, both C2 and C8 prenyl groups are susceptible to modification and both the C2 and C8 prenyl groups gets modified after acid mediated degradation (Figure S8).

2.6. Molecular docking analysis

As illustrated by Nguyen et al. (2014, 2015), AM inhibits biofilm formation of *S. mutans*, a causative agent for dental caries, which predominantly possess Gtf genes. (Xu et al. 2018). AM is also known to inhibit acetylcholinesterase, a key target marker, which alleviates the health condition in Alzheimer's disease as reported earlier. (Chi et al. 2020, Khaw et al. 2014). Hence, in the present study, we intended to determine using molecular docking if AM and DAM has binding affinity for Gtf and acetylcholinesterase. The molecular docking analyses of AM and DAM against glucosyltransferase-SI and acetylcholinesterase demonstrated that, DAM has potential binding energy towards the active sites of glucosyltransferase-SI (GtfC; PDB ID: 3AIC) and acetylcholinesterase (PDB ID: 4EY7) with docking scores of −6.07 and −6.29, respectively, similar to those of AM, with docking scores of −6.09 (glucosyltransferase-SI) and −6.78 (acetylcholinesterase). Also, ligands were docked effectively, with the protein-ligand interaction being within 5 Å. Figures S9 and S10 clearly depicts, the effective H-bond interaction of ligands (AM and DAM) towards the glucosyltransferase-SI and acetylcholinesterase.

Binding free energy is the sum of all the intermolecular interactions that is present between the ligand and the target. Higher the value of free binding energy, the higher the requested energy to break that binding between two molecules, hence, the negative free energy values demonstrate a related binding score between protein and ligand. A higher negative score corresponds to a strong binding and a less negative or even positive score corresponds to a weak or non-existing binding of the ligand with the target. The similar free energy scores of AM and DAM do

indicate that DAM may possess anti-biofilm activity and inhibit acetylcholinesterase if performed in-vitro but may occur at concentration higher than AM as seen for antibacterial activity.

Fauzi and Muchtaridi (2020) in a recent review article describe various derivatives of AM for their effectiveness in treating breast cancer, hence this work, we believe, will open up possibilities of developing alternative strategies for generating better derivatives of alpha mangostin as novel and effective drug candidates for breast cancer, as potent antiviral and antibacterial agents with improved solubility and cell permeability properties.

3. Conclusion

Forced degradation studies helps in determining the degradation of AM in acidic conditions leading to the formation of a structurally different compound (DAM), which had a modified structure as confirmed by NMR and mass spectroscopic analysis. The structural elucidation and antibacterial data presented in the study reveals that the position of the prenyl group at C2 and C8 positions in AM are essential for its potent antimicrobial activity, and its antimicrobial potency is reduced upon acid exposure. However, the degraded compound DAM did not lose its biological activity completely, as proved by the molecular docking studies. The molecular docking results revealed comparable binding energies between DAM and AM with the target proteins, Glucosyltransferase-SI and Acetylcholinesterase, despite reduced antibacterial activity of DAM. Although, based on binding energies in molecular docking studies, DAM shows binding activity similar to AM; it may occur that DAM might show antibacterial activity against *S.mutans* at a concentration higher than AM if performed in-vitro. This intriguing aspect of DAM's behaviour after degradation raises fundamental questions about its potential to affect different biological pathways, even after structural transformation. Thus, forced degradation studies are essential to understand the degradation pathways and products formed upon degradation of the drug substance during exposure to stressed environments; further helping in the development of stress resistant and more stable formulation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Author contributions

The authors and their contributions to the study are as follows. Pratixha Palkar performed initial experiments on AM isolation and its forced degradation studies. Shital Palghadmal ideated the

concept, purified the AM and DAM molecules, carried out their TLC and HPTLC studies and interpreted the data. Shankar Mane and Priti Darne carried out all the microbial experiments. Shankar Vidhate processed the samples for reconfirmation of the LC-MS and NMR data of AM and DAM and carried out the NMR data interpretation. Nisha Mehta prepared a preliminary draft of the manuscript, formatted the figures as per journal's requirement, carried out data analysis and interpretation. Sriram Padmanabhan conceived and designed the research study, supervised, reviewed and edited the manuscript.

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