BIOASSAY-GUIDED ISOLATION OF FURIN INHIBITORS FROM LEAF EXTRACT OF *Momordica charantia* L.

Miss. Oladoyin Grace Famutimi,
Obafemi Awolowo University, Ile-Ife, Nigeria
famutimioladoyin@gmail.com, +2348163749198
ORCID NO: 0000-0002-0135-3727
Prof. Isaac Olusanjo Adewale,
Obafemi Awolowo University, Ile-Ife, Nigeria
olusanjo2002@yahoo.co.uk, +2348038271013
ORCID NO: 0000-0001-8410-4680
Miss. Oghenesivwe Osiebe,
Obafemi Awolowo University, Ile-Ife, Nigeria
osiebekaro@gmail.com, +2347036719985
ORCID NO: 0000- 0002-2823-0831
Prof. Mutalib Adeniran Aderogba,
Obafemi Awolowo University, Ile-Ife, Nigeria
marogba@oauife.edu, +2348035815107
ORCID NO: 0000-0002-2561-8099

ABSTRACT

Proprotein convertase-furin is involved in numerous physiological and pathogenic processes, such as viral propagation, bacterial toxin activation, cancer, and metastasis. Because of its involvement in these disease-related processes, the inhibition of this enzyme could be a promising drug target. The several existing synthetic inhibitors of furin are associated with side effects. Hence, we focused on natural sources, in particular, medicinal plant with antiviral capabilities. This study was designed to isolate the bioactive secondary metabolites present in *Momordica charantia* leaf extract and determine their potential bioactivity on furin.

M. charantia leaves were air-dried, ground to fine powder and extracted using 80% (v/v) methanol. The methanolic extract was concentrated *in vacuo* at 40 ^{o}C and the crude extract obtained was partitioned successively with n-hexane (HEX), dichloromethane (DCM), ethyl acetate (EtOAc) and n-butanol (BuOH). A bioassay-guided screening of the crude extract and solvent fractions was carried out against the activity of recombinant human furin with the release of fluorescent 7-amino-4-methyl coumarin (AMC) liberated from the substrate, Pyroglutamic acid-Arg-Thr-Lys-Arg-methyl-coumaryl-7-amide, in a fluorimeter plate reader. Thereafter, the most potent fraction was subjected to chromatographic separation using thin layer (TLC) and column chromatographic techniques. The eluted fractions and subfractions were screened for bioactivity.

The crude extract showed inhibition percentages of 51.9 and 100% at 7 and 12.5 ng/µl, respectively. The HEX fraction (7 ng/µl) exerted the highest inhibition (72%) on furin compared to other fractions while the BuOH fraction activated the enzyme by 1.5%. The chromatographed HEX fraction yielded seven (7) fractions with different physical properties. Five of these fractions gave single spot on TLC plate. Six fractions (MC I to VI) exhibited potent inhibition against furin with inhibition percentages ranging from 67 to 100% when 0.5 ng/µl of the inhibitor was used. Further fractionation of MC VI on preparative thin layer chromatography gave two sub fractions (A and B) which gave 50% inhibition, respectively.

Overall, the presence of these potent inhibitors of furin in the leaf extract of *M. charantia* could provide a rationale for the ethnomedicinal use of the plant for viral infection in Nigerian folk medicine. Also, further investigations are underway for a better understanding and structural elucidation of the secondary metabolites responsible for the bioactivity observed on furin.

Keywords Bioassay-guided fractionation, Furin, Inhibition, *M. charantia*