







Kinetics of trypsin inhibition by methanolic and solvent-partitioned fractions of two medicinal plants – *Momordica charantia* and *Xylopiya aethiopia*

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Highlights

- The methanolic crude extract of both plants are inhibitor of bovine trypsin.
- The most active trypsin inhibitors of both plants are probably non-polar compounds.

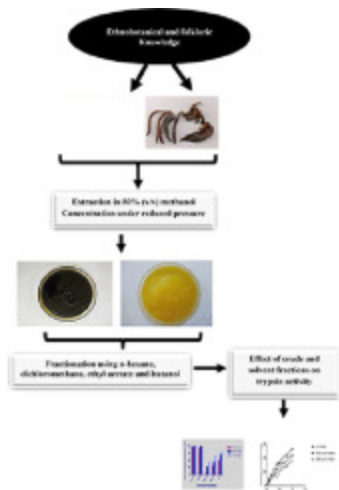
- The K_i of the n-hexane fractions is far lower than that of the methanolic extracts.
- The inhibitors bind at different sites on the trypsin molecule and may be synergistic.

Abstract

Aberrant expression of trypsin has been implicated as part of the overall interplay of biochemical processes responsible for the progression of some diseases. Altering the *in vivo* activity of this enzyme could therefore be targeted for the development of new therapeutics. This study investigated *in vitro* trypsin inhibition properties of *Momordica charantia* (bitter gourd) and *Xylopiya aethiopyca* (Guinea pepper) extracts which are traditionally used in disease management. The leaves of *M. charantia* and fruits of *X. aethiopyca* were extracted with 80% methanol (v/v) to obtain the crude methanolic extract which were further fractionated with solvents of increasing polarity to obtain n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous fractions. Their effect on trypsin activity were thereafter investigated. Crude methanolic extract of *X. aethiopyca* has a higher inhibitory effect on trypsin ($IC_{50}=0.3163\pm 0.02721$ mg/ml) than the crude methanolic extract of *M. charantia* ($IC_{50}=0.8656\pm 0.05855$ mg/ml) whereas the n-hexane fraction of *M. charantia* appears to be more potent ($IC_{50}=0.1017\pm 0.0022$ mg/ml) than the n-hexane fraction of *X. aethiopyca* ($IC_{50}=0.1388\pm 0.00807$ mg/ml). The crude extracts of *M. charantia* acts as an uncompetitive inhibitor of trypsin while the n-hexane fraction acts as a competitive inhibitor. Both the crude extracts and n-hexane fractions of *X. aethiopyca* act as uncompetitive inhibitors.

Hence, the inhibitory activities of *M. charantia* and *X. aethiopyca* suggest that they contain inhibitors of trypsin which could be further developed into compounds capable of modulating activities of trypsin.

Graphical abstract



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Introduction

Trypsin has been reported to be involved in different types of cancer in the process of angiogenesis / proliferation, invasion and metastasis in the entire carcinogenic processes. The aggressiveness of tumors has been found to be affected by the overexpression of trypsin (Soreide et al., 2006). Studies have shown that deficiency of the pancreatic secretory trypsin inhibitor (PSTI) in the pancreas leads to the autoactivation of digestive enzymes in the pancreas and then ultimately the digestion of the pancreas. This is the main causative event of the onset of pancreatitis (Hirota et al., 2006r"?>t"?>). Severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2), closely related to SARS-CoV-1 is a novel coronavirus identified as the causative agents of respiratory diseases in January 2020. In cultured cells, Kim et al. (2022) revealed that trypsin enhances the replication of SARS-CoV-2 and also facilitate the viral entry by promoting a direct fusion process at the cell surface (Kim et al., 2022).

Trypsin can be considered as a target for the development of new potent therapeutics therefore, inhibitors of this enzyme may have considerable prospect as therapeutic agent in the management of the diseases in which aberrant trypsin activity is involved. Some medicinal plants have been employed traditionally in the treatment of some of these disease conditions and are rich source of pharmaceutically active phytochemicals, peptides and proteins responsible for their optimum therapeutic efficacy. Thus, it is worth evaluating their inhibitory property on trypsin (Bijina et al., 2011). Inhibition of enzymatic activities by medicinal plant extracts are now known to be an integral part of pharmacological actions of herbal medicines. For example, Mahomoodally et al. (2018) showed that extracts of *Aegle marmelos* contain essential oil such as limonene, β -phellandrene, *p*-cymene and β -caryophyllene which are inhibitors of tyrosinase, α -glucosidase and acetylcholinesterase. Some of these enzymes are implicated in the progression of some diseases.

Momordica charantia also known as bitter melon, bitter gourd, karela, balsam pear, belongs to the family Cucurbitaceae and is widely used as traditional medicine in most parts of the world. It is widely distributed in Asia, South-America and Africa (Basch et al., 2003; Li et al., 2020). *M. charantia* has been traditionally used for treatment of human diseases such as diabetes, malaria, cancer, peptic ulcer and infectious diseases (Khurshid et al., 2020). A lot of medicinal properties of *M. charantia* have been studied that include antiviral, antioxidant, antibacterial, antitumor, antidiabetic and anti-inflammatory. *In vitro* studies have shown that proteins in *M. charantia* (α - and β -monocharin) have inhibitory effect on human immune deficiency virus (HIV) (Saeed and Tariq, 2005 and Tariq?>; Jia et al., 2017). Extracts from *M. charantia* are effective in lowering blood fat content and the level of blood glucose in diabetic and obese rodents fed with a high fat diet (Grover and Yadav, 2004; Khurshid et al., 2020). The ethanol extract of leaf and stem of *M. charantia* has been reported to inhibit the growth of *E. coli* and *Staphylococcus aureus* (Wang et al., 2017). Although, *Momordica charantia* possesses several pharmacological activities, it has been reported to have some adverse effects in the past years which reduce its wider herbal application. Previous studies confirmed that it induces symptoms of abortion or even death of laboratory animals and hypoglycemic coma in children (Grover and Yadav, 2004; Jia et al., 2017).

Xylopi aethiopic a is a slim tree about 60–70cm in diameter, 15–30m tall, mainly found growing in the tropical forest of Ghana, Nigeria and Cameroon. It belongs to the Annonaceae family (Burkill, 1985; Obiri et al., 2014). The fruits of *Xylopi aethiopic a* are kidney shaped, small, and twisted which are dark brown, cylindrical of 2.5cm to 5cm long and 4 to 6mm thick (Orwa et al., 2009). Its common names include African pepper, Guinea pepper, negro pepper, west African and Senegal pepper (Jirovetz et al., 1997). Studies have proved it to be useful in the treatment of diarrhea, dysentery, stomach disorder, menstrual disorder, naso-pharyngeal infections, arthritis, rheumatism, viral infections, among others (Erhirhie and Moke, 2014). The fruits are useful in the treatment of bronchitis, asthma, stomach aches and dysentery (Burkill, 1985). Studies also claimed that intake of *Xylopi aethiopic a* can help to produce a favorable lipid profile in the serum and postmitochondrial fractions (PMFs) of visceral organ in experimental hypercholesterolemia (Nwozo et al., 2011) and that the plant may be a good source of electrolyte balance (Johnkennedy et al., 2011). Adaramoye et al. (2010) investigated the effects of *Xylopi aethiopic a* fruit methanol extract on oxidative stress in brain of rats exposed to whole body gamma-radiation using Vitamin C (VC) as a standard antioxidant. Their findings revealed that treatment with *Xylopi aethiopic a* and VC ameliorated the radiation-induced decreases in antioxidant status of the animals. These suggests that *Xylopi aethiopic a* could have beneficial effect by inhibiting oxidative damage in the brain of exposed rats.

This current study aimed to investigate the *in vitro* trypsin inhibitory activities of methanolic extracts and solvent-partitioned fractions of *Momordica charantia* leaves and *Xylopi aethiopic a* fruits as potential source of therapeutic agents in the management of some diseases.

Section snippets

Materials

All chemicals and reagents were of analytical grade. Purified trypsin from bovine pancreas was a product of Boehringer-Mannheim, GmbH, Germany. N_{α} -benzoyl-DL-arginine-4-nitroanilide (BAPNA) used as substrate was a product of Sigma Chemical Company, St. Louis, USA....

Plant material and extraction procedure

Fresh plants of *Momordica charantia* were obtained within Obafemi Awolowo University, Ile-Ife campus in Nigeria and *Xylopi aethiopia* fruits were purchased from Ita-Akogun market, Ile-Ife. After authentication at the Herbarium of the...

Solvent fractionation of phytoconstituents of *M. charantia* and *X. aethiopia*

The percentage yield of methanolic extract was based on the weight of dried plant materials with both plant materials having the same yield of extraction (12%). The percentage yield of the solvent-solvent fractionation of the methanolic extract of the two plants ranged from 0.5 – 29% (0.47 – 30.55g) (Scheme 1). The results obtained showed that more polar phytochemicals are present in the *M. charantia* extract than non-polar compounds and more non-polar compounds than polar compounds for X...

Conclusion

In conclusion, both the methanolic crude extract of *M. charantia* and *X. aethiopia* contain phytoconstituents which are inhibitors of bovine trypsin. Solvent fractionation partitioned the inhibitors into various fractions with the n-hexane fractions exhibiting the highest antitrypsin activity followed by dichloromethane fractions. Thus, the most active trypsin inhibitors of the two medicinal plants are probably non-polar compounds. Inhibition constant, K_i is a measure of inhibitor potency. The...

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Declaration of Competing Interest

The authors have no conflicts of interest to disclose....

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