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Original Article

Inhibitory potentials of *Moringa oleifera* on activities of neuraminidase, xanthine oxidase and adenosine deaminase

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ABSTRACT

Background and Aims: The use of *Moringa oleifera* as nutraceuticals in alternative medicine has received tremendous attention in recent years. Its diverse bioactive composition, multipurpose benefits and ease of cultivation give it a superior advantage over other herbs.

Methods: Fresh leaves and roots were obtained from *M. oleifera* grown in northwestern Nigeria. The inhibitory effect of *M. oleifera* extracts on the activities of neuraminidase, xanthine oxidase, and adenosine deaminase were determined.

Results:: The present study explored the aqueous, methanol, and hexane extract of *M. oleifera* leaves and roots for the inhibition of neuraminidase, xanthine oxidase, and adenosine deaminase. In comparison to quercetin (Half maximum inhibitory concentration (IC_{50}) = 14.28 ± 2.30 µg/mL), aqueous (IC_{50}= 0.12 ± 0.01 µg/mL) and methanol (IC_{50} = 0.57 ± 0.13 µg/mL) the extract of the moringa root strongly inhibited neuraminidase activity. The enzyme was moderately inhibited by aqueous (IC_{50} = 89.56 ± 9.77 µg/mL) and hexane (IC_{50} = 104.33 ± 3.39 µg/mL) extracts of the plant leaf. The inhibition of xanthine oxidase by aqueous (IC_{50} = 7543.86 ± 1127.19 µg/mL), and methanol (IC_{50} = 1779.48 ± 126.50 µg/mL) leaf extracts were far below that of a standard inhibitor - allopurinol (IC_{50} = 0.88 ± 0.01 µg/mL). Amongst the extracts used, only the hexane extract of the moringa leaf (IC_{50} = 4580.38 ± 75.69 µg/mL) inhibited adenosine deaminase and was less effective than erythro-9-(2-Hydroxy-3-nonyl)-adenine hydrochloride (EHNA) (IC_{50} = 53.00 ± 1.83 µg/mL).

Conclusion: The findings suggest that moringa roots and leaves can be an excellent source of agents against microbial infection and viral induced respiratory syndrome. The extracts may also attenuate influenza A infection, the progression of oxidative stress, cancer, inflammation, diabetes, cardiac failure, and coronary artery disease, since they have an effect on neuraminidase, xanthine oxidase, adenosine deaminase, and possibly superoxide levels.

Keywords: *Moringa oleifera,* Neuraminidase, Xanthine oxidase, Adenosine deaminase, Inhibition.

INTRODUCTION

Many drugs exhibit biochemical and clinical effects via hindering the activity of enzymes (biological catalysts), either by directly blocking the binding of substrates to enzyme (thereby preventing enzyme-complex formation), or by retarding the catalytic activity of an enzyme rate (i.e., rate of product formation) upon binding to a regulatory/allosteric site. Therefore, many drugs function as inhibitors of enzymes. Thus, enzyme inhibition is amongst the principal techniques employed for drug discovery. This technique and regimen have paramount importance in both therapeutics and pharmacognosy. This is due to the widespread use of plants (as alternative or folk medicine) in the treatment or management of metabolic diseases, metabolic disorders, infectious diseases, drug resistant strain of microbes, and pathogens, in addition to their use as nutraceuticals and food additives (Hodas, Zorzenon, & Milani, 2021).

Neuraminidases are a group of glycoside hydrolases that catalyze the hydrolysis of the glycosidic bonds of neuraminic acid and/or its derivative (sialic acids). These enzymes play a significant role in microbial pathogenesis and virulence (Rothe, Rothe, Roggentin, & Schauer, 1991). They are believed to modulate motility, virion aggregation, elution of virion progeny as well as the interaction between pathogens and host cell receptors (McAuley et al., 2017). Therefore, inhibition of these group of enzymes confers great advantage to host organisms against the virulent and infectious agents as earlier demonstrated by Gulati et al. (2013).

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Xanthine oxidase is an important xanthine oxidoreductase involved in purine metabolism. It oxidizes hypoxanthine to xanthine, and then to uric acid. This reaction is accompanied by the generation of superoxide radicals (Rechreche, Abbes & Iovanna, 2020), thus assuming great significance in the antioxidant system. The activity of xanthine oxidase in reported to increase in several disease conditions. These include oxidative stress, diabetes, cardiac failure, coronary arteries disease, and in influenza A infection (Penislusshiyan, Chitra, Ancy, Kumaradhas, & Palvannan, 2020). Therefore, inhibition of this enzyme is of great significance in attenuating the accumulation of a reactive oxygen species and pathogenesis/progression of many disease conditions.

The irreversible deamination adenosine to inosine in purine metabolism is catalyzed by adenosine deaminase. This same enzyme has demonstrated to be critical for immune responses, transmission of nerve impulses, pregnancy, and the differentiation of epithelial cells (Moriwaki, Yamamoto, & Higashino, 1999). Elevated levels of adenosine deaminase are reported in arthritis, psoriasis, sarcoidosis, cancer, ischemia, haemolytic anemia, and AIDS (Blackburn & Kellems, 2005). Therefore, food-based extracts/compounds capable of diminishing adenosine deaminase activity will play a vital role in the management of diseases and their accompanying symptoms.

In recent years, *Moringa oleifera* received a tremendous amount of attention in the field of alternative medicine, either as nutraceuticals, food supplements, or herbs (in the form of tea or spices) due to its diverse bioactive composition, benefits, and ease of cultivation. A review by Pandey et al., (2012) and more recently by Khor, Lim, Moses & Abdul Samad, 2018, advocates that *M. oleifera* exhibited several medicinal properties. These reports indicated that the plant exhibited antidiabetic, antihypertensive, anticancer, antioxidant, anti-inflammatory, antipyretic, antiplasmodial, and antimicrobial effects, in addition to chemoprotective and radioprotective action properties. In addition, the extracts are cytotoxic to a diverse type of cancer cells but had minimal toxicity to normal cell and experimental animals. The plethora of multifarious therapeutic effects of *M. oleifera* is attributed to its disparate and assorted chemical or phytochemical composition (Ajagun-Ogunleye & Ebuehi, 2020). The aim of the present study is to investigate the inhibitory potentials of *M. oleifera* leaves and roots extracts on neuraminidase, xanthine oxidase, and adenosine deaminase.

MATERIALS AND METHODS

Sample collection and preparation

The fresh leaves and roots of *M. oleifera* were obtained from Northwest Nigeria. The plant was authenticated by a Taxonomist (Umar Abdullahi, PhD), at the Botany Unit of Biological Sciences Department, Usmanu Danfodiyo University Sokoto. This was followed by deposition of a voucher specimen (Voucher Number: UDUS/VS/2011/31) in the University herbarium. The plant samples were processed, and extracts prepared according to the method of Magaji, Sacan, & Yanardag, (2020).

Enzyme inhibition assay

The inhibitory effect of *M. oleifera* extracts on the activities of neuraminidase, xanthine oxidase, and adenosine deaminase were determined according to the method of Myers et al., (1980), Abdullahi et al., (2012) and Blum & Schwedt, (1998), respectively. Quercetin, allopurinol, and erythro-9-(2-Hydroxy-3-nonyl) adenine hydrochloride (EHNA) were used as standard inhibitors of neuraminidase, xanthine oxidase, and adenosine deaminase. The results are presented as mean ± standard deviation of triplicate values. Half maximum inhibitory concentration (IC_{50}) were calculated from % enzyme inhibition activities using regression analysis data. The IC_{50} values are inversely correlated to inhibition.

RESULTS AND DISCUSSION

The inhibitory activities of aqueous, methanol, and hexane extracts of the moringa leaf and root on neuraminidase are presented in Table 1. The outcome of the present study indicates that both the aqueous and methanol root extracts of moringa exhibited strong neuraminidase inhibitory activity (with IC_{50} values corresponding to 0.12 ± 0.01 µg/mL and 0.57 ± 0.13 µg/mL, respectively). Their inhibitory effect was higher than that of quercetin (IC_{50} = 14.28 ± 2.30 µg/mL), which was used as a standard inhibitor. Moderate inhibition was exhibited by an aqueous extract (IC_{50} = 89.56 ± 9.77 µg/mL) and hexane extract (IC_{50} = 104.33 ± 3.39 µg/mL) of the plant leaf. However, the methanol leaf extract and hexane root extract did not exhibit neuraminidase at the tested concentrations. Fouad, Abu Alnaga, & Kandil, (2019) demonstrated that the moringa leaf extract had a strong antibacterial effect on pyogenic bacteria isolated from the abscess of a dromedary camel. The microorganisms inhibited are *Escherichia coli, Staphylococcus aureus, Micrococcus spp., Citrobacter spp., Corynebacterium pseudotuberculosis, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Corynebacterium ulcerans,* and *Staphylococcus epidermidis*. In another study by Dahot (1998), fractions of moringa extracts were shown to inhibit bacteria *(E. coli, Klebsiella aerogenes, K. pneumoniae and Bacillus subtilis)* and *Aspergillus niger* (a fungus). In comparison to amoxicillin, the moringa leaf extracts were shown to be a better antibiotic candidate against Bacillus spp. (Kilany, 2016). The antimicrobial studies by Elgamily et al. (2016) revealed that both root and leaf extracts of the moringa significantly inhibited the growth of *S. aureus* and *Streptococcus mutans*, but had no effect on *Candida albicans* growth. These reports agree with the present findings, where the leaves and roots demonstrated anti-neuraminidase

Table 1. Inhibitory effect of *M. oleifera* extract on neuraminidase activity.

Enzyme	Extract/ Standard	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Neuraminidase	Aqueous leaf extract	400.00	79.61 ± 4.87	89.56 ± 9.77
		200.00	70.62 ± 0.48	
		100.00	53.44 ± 0.74	
		10.00	35.29 ± 1.47	
	Methanol leaf extract		ND	
	Hexane leaf extract	100.00	45.12 ± 1.41	104.33 ± 3.39
		50.00	38.36 ± 0.59	
		30.00	21.80 ± 2.56	
		20.00	12.89 ± 1.86	
	Aqueous root extract	0.10	43.58 ± 2.39	0.12 ± 0.01
		0.08	34.74 ± 0.09	
		0.05	11.62 ± 1.59	
		0.01	5.40 ± 0.18	
	Methanol root extract	1.00	70.72 ± 1.63	0.57 ± 0.13
		0.75	58.43 ± 2.83	
		0.05	14.24 ± 1.43	
		0.01	6.44 ± 2.36	
	Hexane root extract		ND	
	Quercetin	70.00	91.62 ± 0.63	14.28 ± 2.30
		40.00	77.05 ± 1.09	
		20.00	55.56 ± 2.28	
		10.00	42.23 ± 2.42	

* = Mean ± SD of triplicate values; ND = Activity not detected.

activity (an enzyme necessary for microbial pathogenesis, virulence, and motility). The stronger neuraminidase inhibition by the moringa root extract may be attributed to its elevated levels of 4-(alpha-lrhamnopyranosyloxy) benzylglucosinolate and benzyl glucosinolate than was reported in the leaves (Bennett et al., 2003). Quercetin, kaempferol, and myricetin are flavonoids found in moringa leaves (Athira Nair & James, 2020). These compounds and their derivatives were demonstrated to inhibit both 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) (Athira Nair & James, 2020; Jo, Kim, Shin, & Kim, 2020) – the two main protease enzymes critical for the virulence of several viruses including severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). A recent review revealed that quercetin (a compound chiefly available in the moringa) was one of the most potent compounds with anti-CoV activity (Solnier & Fladerer, 2020). Thus, moringa extracts can be indispensable antibacterial and antiviral agents due to their neuraminidase, 3CLpro and PLpro inhibition properties.

As seen in Table 2, only aqueous (IC_{50} = 7543.86 ± 1127.19 µg/mL), and methanol (IC_{50}= 1779.48 ± 126.50 µg/mL) leaf extracts of the moringa had an inhibitory effect on xanthine oxidase in the present study. The effect of the extracts was much lesser than that of allopurinol (IC_{50} = 0.88 ± 0.01 µg/mL). Yumita, Suganda, & Sukandar, (2014) reported that the root of the moringa exhibited xanthine oxidase. This contrasts with the findings of the present study where only the leaf extracts had xanthine oxidase inhibition.

As shown in Table 3, the inhibitory effect of the hexane extract of the moringa leaf (IC_{50} = 4580.38 ± 75.69 µg/mL) on adenosine deaminase activities was low as compared to that of EHNA (IC_{50} = 53.00 ± 1.83 µg/mL). Though not many reports are available on the effect of moringa on adenosine deaminase activity, the aqueous extract of plants such as Urtica dioica have been shown to strongly inhibit adenosine deaminase of prostate tissue (Durak, Biri, Devrim, Sozen, & Avci, 2004). What is more, several studies have shown that moringa extracts

Table 2. Inhibitory effect of *M. oleifera* extracts on xanthine oxidase activity.

Enzyme	Extract/ Standard	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Xanthine oxidase	Aqueous leaf extract	4000.00	31.88 ±2.66	7543.86 ± 1127.19
		3000.00	25.18 ± 3.91	
		2000.00	18.94 ± 1.25	
		1000.00	15.69 ± 0.59	
	Methanol leaf extract	3000.00	73.80 ± 3.78	1779.48 ± 126.50
		2000.00	51.75 ± 0.78	
		500.00	29.05 ± 3.26	
		250.00	20.10 ± 2.14	
	Hexane leaf extract		ND	
	Aqueous root extract		ND	
	Methanol root extract		ND	
	Hexane root extract		ND	
	Allopurinol	2.00	98.79 ± 0.24	0.88 ± 0.01
		1.00	82.50 ± 1.59	
		0.50	25.23 ± 1.82	
		0.25	5.87 ± 1.58	

* = Mean ± SD of triplicate values; ND = Activity not detected.

Table 3. Inhibitory effect of *M. oleifera* extract on adenosine deaminase activity.

Enzyme	Extract/ Standard	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Adenosine deaminase	Aqueous leaf extract		ND	
	Methanol leaf extract		ND	
	Hexane leaf extract	5000.00	54.54 ± 1.53	4580.38 ± 75.69
		4000.00	41.94 ± 0.28	
		3000.00	34.17 ± 1.27	
		2000.00	15.56 ± 1.11	
	Aqueous root extract		ND	
	Methanol root extract		ND	
	Hexane root extract		ND	
	EHNA	60.00	52.78 ± 0.80	53.00 ± 1.83
		40.00	44.63 ± 0.20	
		20.00	39.48 ± 1.14	
		10.00	34.94 ± 0.49	

* = Mean ± SD of triplicate values; ND = Activity not detected.

have anticancer and cytotoxic effects (Jung, 2014; Khor et al., 2018). Thus, the inhibition of this enzyme, coupled with the antioxidant activity of the moringa extract may not be unrelated with its anticancer, anti-inflammatory as well as tissue protective effects.

CONCLUSION

The outcome of the present study suggests that root and leaf extracts of the *M. oleifera* have promising anti-neuraminidase, and are a suitable candidate for new and effective antimicrobial agents including influenza A and corona viruses. The inhibition of xanthine oxidase and adenosine deaminase by leaf extracts suggest that the plants can be a source of compounds that can be used to manage or attenuate the progression of oxidative stress, cancer, inflammation, diabetes, cardiac failure, coronary arteries disease, and viral induced respiratory syndrome disease.

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