J. Chem. Soc. Nigeria, Vol. 46, No. 6, pp 1062 – 1067 [2021]

PHYTOCHEMICAL SCREENING, ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITIES OF FRACTIONATED ETHANOLIC EXTRACT OF *Moringa Oleifera* LEAVE

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ABSTRACT

Moringa oleifera leave has been reputed for the treatment of different ailments, this work thus aims at isolating the active principle(s) that are responsible for these activities. *M. oleifera* leave was extracted with distilled ethanol, concentrated to afford the crude extract (210.2 g). The crude extract was partitioned using n – hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) in an increasing order of polarity to afford 12 fractions. The fractions were subjected to biological testing and MOF8 and MOF11 were selected for further purification based on their antioxidant and antimicrobial activities. The two fractions (MOF8 and MOF11) were purified on a silica gel open column, using NH, DCM, EA and MeOH in an increasing order of polarity to afford ten and eight fractions respectively. Further biological test on these fractions indicates superior activities of $MOF11_8$ which was further purified as described earlier to afford four fractions. Further biological testing on these fractions indicates that and MOF118.3 and MO118.4 are more active in the antioxidant and antimicrobial analysis. Phytochemical screening of MOF11 and MOF11 indicates the presence of flavonoids, saponin, tannins, alkaloids, glycosides and steroids.

Key words: Extraction, fractionation, chromatography, isolation, antioxidant, antimicrobial.

INTRODUCTION

Moringa Oleifera is a deciduous tree, long-lasting, short, thin with hanging branches, develops to around 10 meters in stature; stem and branches are fragile; leaves are light green, fluffy with numerous little pamphlets, stamens are yellow; flowers are fragrant, white or velvety white; pods are triangular, brown containing around 20 seeds implanted in the pith. The tallness of Moringa plant can achieve 12 m with a trunk of 30 cm wide [1].

The extracts of different parts Moringa such as seeds cotyledon, seeds coat, stem bark, leaves, root bark have been reported to possess antimicrobial potential [2]. In the study of Singh and Sharma [3], the antimicrobial activity of *Moringa oleifera* was examined using the main model Kirby-bauber disc diffusion method in which 50% of ethanolic Moringa leaf extract was used. The results showed that the 50% ethanolic extract successfully exhibit moderate anti-bacterial activity. Even at higher concentrations, the extract displayed mild inhibitory activity and no activity at all against pseudomonas. The antibacterial activity of the Moringa extract was observed to be greater against gram-positive species (*S. aureus* and *E. faecalis*) than against gram-negative species (*E. coli*, *Salmonella*, *P. aeruginosa*, *V. parahaemolyticus* and *A. caviae*) which was also indicated in several other studies [4 – 6].

The antioxidant properties of *Moringa oleifera* was examined by Verma et al. (2012) in which 50% ethanolic leaf extract was tested to investigate the lipid peroxidation (LPO),

ISSN 0795 - 22066

catalase (CAT) and superoxide dismutase (SOD) activities. The antioxidant properties of the Moringa extract was found to change in SOD, CAT and LPO levels in rat gastric mucosa. There was a reported increase in gastric mucosal SOD and LPO activities during ulcer conditions which indicated an antioxidant defense by the *Moringa oleifera* extract [7]. Similarly, another study by Siddhuraju and Becker [8] using DPPH assay to determine the antioxidant activity, revealed that the extracts of Moringa leaf samples from three different agroclimatic origins had very high radical scavenging activity. It was stated that generally, the higher the total polyphenols, the higher the antioxidant activity, in which it is most likely to be due to the combined action of several existing substances as well as the high hydrogen donating ability. In addition, the study also used several other methods to assess the antioxidant activity of the Moringa extracts, whereby all other methods likewise demonstrated the same antioxidant activity in terms of reducing potassium ferricyanide, scavenging superoxide radicals, preventing peroxidation of lipid membranes in liposomes, inhibiting oxidation of microsomes in rat liver, inhibiting the peroxidation of linoleic acids and preventing bleaching of carotenes.

According to Sreelatha and Padman [9], qualitative analysis of the extract was conducted to identify the chemical nature of the active compounds which were possibly providing these antioxidant protection properties, this reveals the presence of phenolics, flavonoids and trace amounts of alkaloids in both mature and tender leaves. A high content of γ-tocopherol have been found in practically the whole plant, ranging from 5.7 µg/g (adult leaves) to 27.8 µg/g (6 month-old leaves) of dry mass. Important variations have been encountered in α-tocopherol values which ranged from 95.9 µg/g (green seeds) to 744.5 µg/g (adult plant leaves) [10], differences probably explained by the variation between the age of the plants and their varied parts. Vitamin E is an antioxidant substance which is basically composed of tocopherols; however, α-tocopherol is the most active and abundant constituent in *M. oleifera*, [11]. These antioxidant substances metabolize peroxides before they can cause any injury to cell membranes, maintaining the intracellular redox status. Violation of this status favors oxidative stress, resulting in pathologic manifestations, such as atherosclerosis and cancer, [12].

From the foregoing, we intend to investigate and establish the chemical compound(s) responsible for these activities in *Moringa oleifera* leave grown in Ilorin, North Central Nigeria following biological activities guided isolation protocols.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

Moringa Oleifera leave was obtained from the moringa plantation, University of Ilorin, Ilorin. The leaves were air dried, grinded, weighed and kept until further study. A known amount (4.0 Kg) of the grinded leaves was extracted ethanol. The extract was concentrated, weighed and stored in labeled sample bottle until further analysis.

Fractionation and Isolation

Solvent portioning and chromatography were employed for the fractionation and isolation of the samples. The crude extract (210.2 g) of *Moringa Oleifera* leave was partitioned using n – hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) in an increasing order of polarity to afford 12 fractions. The column chromatography (CC) was carried out on silica gel (60 – 200 mesh size, Merck, Germany). Thin Layer Chromatography (TLC) was carried out on pre – coated silica gel 60 F_{254} aluminum foil (Merck, Germany) for the establishment of the purity of isolates. Spots on TLC were examined with a UV lamp operating at a wavelength of 366 nm for fluorescence and at 254 nm for fluorescence quenching spots.

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Phytochemical Screening

The phytochemical analysis was carried out on fraction MoF8 and MoF11 to ascertain the various classes of organic compounds present in the fractions. Standard phytochemical procedures for qualitative detection of alkaloids, flavonoids, tannins saponins, steroids and glycosides according to the methods of Harbone [13], Trease and Evans [14]) and Kokate [15] were adopted.

Biological Studies

The antimicrobial screening and antioxidant activities of the extract, fractions and isolates were carried out at the Department of Microbiology, University of Ilorin, Ilorin.

Antimicrobial Analysis:

The samples were dissolved in 10% dimethyl sulfoxide (DMSO) and were stored at 4^0C. The antimicrobial activity was tested against two selected bacterial; *Eschericia coli* and *Salmonella typhi*, according to agar plug diffusion method as described by Balouiri *et al*., [16]

Antioxidant Analysis: DPPH Method

The antioxidant activities of the samples were estimated using 2,2, - diphenyl – 1-picrylhydrazyl (DPPH) assay. The DPPH was used as free radical and ascorbic acid used as standard to assess the inhibitory activity based on established experimental procedures [17 – 19].

RESULTS AND DISCUSSIONS

Over the past years, numerous experimental and epidemiological studies have shown that a wide variety of plant materials have several biological properties. Natural products mainly from the plant kingdom among which we have *Moringa oleifera*, offer a wide range of biologically active compounds that act as natural antioxidants with potentials in drug discovery and development [19]. *M. oleifera* leaves contain abundant glucosinolates, isothiocyanates, carotenoids, vitamins, high proteins, and carbohydrates [21], hence, the leaves have been promoted as components of foods, nutritional supplements, or medicines for patients with HIV/AIDS by several African governments [22] and as supplement to maize traditional complementary food to improve iron status in infants in Nigeria [23]. This study has established a protocol for biologically guided isolation of active components of *M. oleifera* leave ethanol extract. The DPPH scavenging and antibacterial activities have been employed for the isolation.

Preliminary investigation of phytochemical constituents of the extracts showed the presence of alkaloids, flavonoids, steroids, glycosides, tannins and saponins in MOF8 and MOF11 fractions (Table 1). This result is slightly different from those of Ukachi et al, [24] who confirmed the presence of flavonoids, terpenoids, phenols and carbohydrates in the methanol and ethyl acetate extracts of *M. oleifera*.

Table 1: showing presence of metabolites where (+) denotes positive and (–) denotes negative

Fractions	Metabolites					
	Alkaloid	Flavonoid	Steroid	glycoside	Tannins	Saponin
MOF8	+	+	-	+	+	+
MOF11	+	+	+	+	+	+

Figures 1 and 2 present the antioxidant activities of the various samples, this was also described by Tatiana *et al.*, 2013 which showed high antioxidant activity and also exhibit high antimicrobial activities against Candida albicans and gram positive bacterial

ISSN 0795 - 22066

but weak activities in gram negative bacterial. All the fractions exhibited high antioxidant potency (Figure 1), but this research work focuses on the fractions that exhibit both antioxidant and antimicrobial activities. The isolates from this fraction has high antioxidant activities but $MOF11_{8,3}$ and $MOF11_{8,4}$ possesses higher antioxidant and antimicrobial activities.

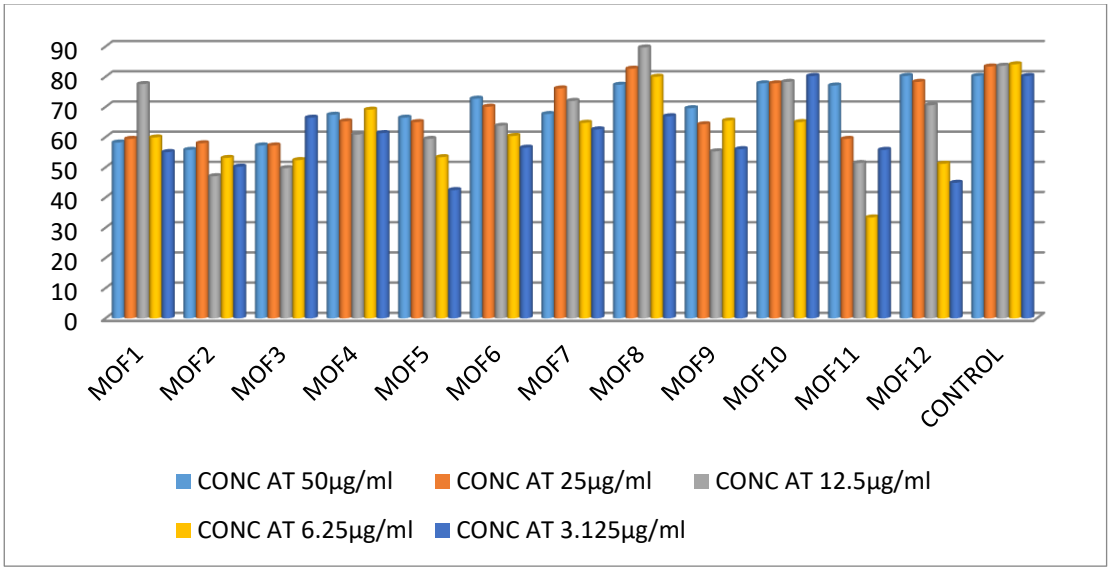

Fig 1: Percentage scavenging effect of fractionated ethanolic extract of *Moringa oleifera* leave.

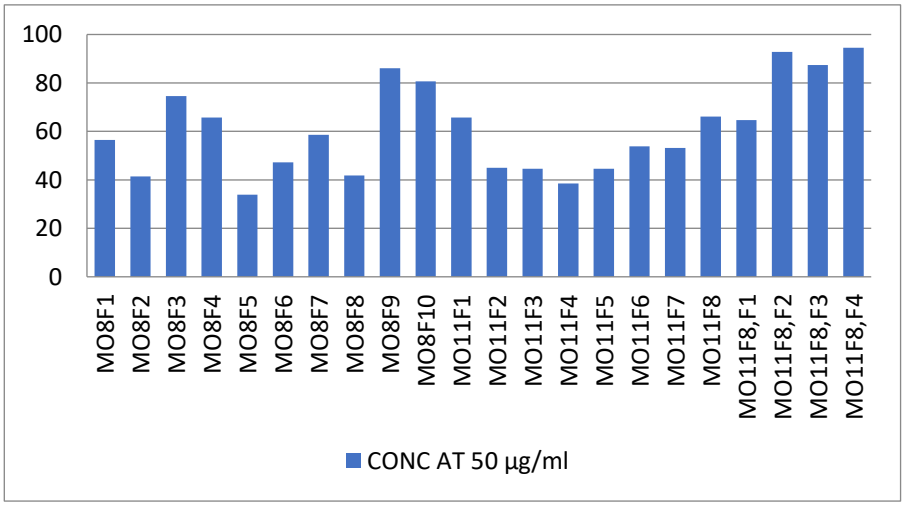

FIG 2: Percentage scavenging effect of purified MoF8, MoF11 and MoF11,f8 fractions of *Moringa oleifera* leave

Table 2: Antimicrobial activities of isolates of *Moringa oleifera* leave ethanol extract.

Samples	Zone of inhibition (mm)	
	Salmonella typhi	*Eschericia coli*
$MOF11_{8,1}$	**9**	**6**
$MOF11_{8,2}$	**6**	**18**
$MOF11_{8,3}$	**20**	**13**

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$MOF11_{8,4}$	**6**	**6**
Control (*streptomycin*)	**16**	**18**

CONCLUSION

In conclusion, this initial investigation has led to activity guided isolation of $MOF11_{8,3}$ and $MOF11_{8,4}$ which are both active in antioxidants and antibacterial tests. Further analyses are being carried out to characterize the isolates and establish their anticancer properties.

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ISSN 0795 - 22066