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COMPARATIVE PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ATTRIBUTE OF THE YOUNG AND THE MATURE LEAVES OF *MORINGA OLEIFERA*

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ABSTRACT

The young and the mature leaves of *Moringa oleifera* were examined for variation in the phytochemical constituents as well as the antimicrobial efficacy of the plant leaves extract. Results obtained from the phytochemical analysis revealed significant difference ($P \leq 0.05$) in the contents of tannin and flavonoids respectively. However, no significant difference ($P \leq 0.05$) unveils in the contents of alkaloids. The antimicrobial analysis revealed that the mature leaves of this plant were more sensitive to the growth of the micro-organism, tested. Findings from this study deduced that the older the plant leaves of *Moringa oleifera*, the higher the phytochemicals, and the better the antimicrobial efficacy.

KEYWORDS: Young leaves, mature leaves, *Moringa oleifera*, phytochemicals, and antimicrobial.

INTRODUCTION

Traditional medicine is a common practice in most part of the West Africa countries, especially among the local communities. *Moringa oleifera* “a unique plant” is widely employed for medicinal purposes. The plant is a very useful breakthrough in the demand of alternative natural medicine for the treatment of various diseases caused by pathogenic organism (Ojiako, 2014). The active compounds present in the leaf of this plant have proven to reduce the incidence of tumors in experimental animals (Bharali *et al*., 2003). Every part of this plant is rich in phytochemicals (Bamishaiye *et al*., 2011). The species of *Moringa oleifera* are well documented plant herb due to their extraordinary nutritional and medicinal properties (Walter *et al*., 2011). The medicinal efficacy of this plant includes anthelminthic, antibiotic, antipyretic, antiasthmatic and analgesic activity (Hukkeri *et al*., 2006: Rao *et al*., 2008: Farooq *et al*., 2012: Thilza *et al*., 2010). *Moringa olefera* is also used as antispasmodic, stimuli expectorant, and diuretic activity (Nadkarni *et al*., 2009).

The leaves of *Moringa oliefera* have been reviewed as an efficient traditional herb for the treatment of diseases caused by micro-organisms. As such, wealth of information is available on the antimicrobial efficacy of the plant leaf extracts. However, no precise information is available on the phytochemical and antimicrobial contrariety of the young and the older leaves of this plant. Hence, the present study is aimed at evaluating the difference in the phytochemical constituents as well as the antimicrobial discrepancy of the young and the mature leaves of *Moringa oleifera*.

MATERIALS AND METHODS

Plants material

The plant materials (young and mature leaves) of *Moringa Oleifera* were obtained from Moringa farm, National Research Institute for Chemical Technology, Bassawa, Zaria, Kaduna state, Nigeria. The plant was reconfirmed as *Moringa Oleifera* at the Department of Biological sciences Ahmadu Bello University (ABU) Zaria, Kaduna state, Nigeria.

Plant preparation

The samples were dried at room temperature for a period of four weeks, and crushed into powdered form using mortar and pestle.

Extraction

About five hundred grams (500g), of the crushed (powdered) Moringa leaves were sieved into fine form, and exactly 50g of each sample was extracted with methanol in 500ml conical flask. The two flasks were covered with Aluminum foil and hanged on a Stuart flask shaker. The two samples were shaken for six hours per day for a period of six days, and allowed to stand for another six days. The extracts were filtered using whatman No.1 filter paper. The solvent (methanol) was recovered using senco rotary evaporator. The extract left in the two flask were exposed to air for complete dryness of the plant extracts.

Phytochemical Screening

The plant secondary metabolites were determined quantitatively using standard procedures. The procedures described in the work of Graceline *et al.*, (2013) were used for the determination of the plant secondary metabolites.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum concentration of the methanolic extract of both samples was determined using the method of (Greenwood, 1989) as described by (Geidam *et al.*, 2007). The dilution of the extract was made serially in concentrations of 250, 200, 150, 100, 50 and 25mg/ml respectively. The concentrations were used to determine minimum inhibition zone of the methanolic extracts of the young and older leaves of this plant.

Micro-Organism

The micro-organisms employed in this study were *Escherichia Coli*, *Stephylococcus aureus*, *pesudomonas aeruginosa*, and *Klebsiella pneumonae*.

RESULTS AND DISCUSSION

Table 1: Some phytochemical constituents in the methanolic extract of the young and the mature leaves of *Moringa Oleifera*.

Phytochemicals	Young leaves	Mature leaves
Tannin (g/100g)	$15.26\pm0.21^{**}$	$25.84\pm0.61^{*}$
Saponin (g/100g)	0.96±0.04	1.83±0.03
Flavonoid (g/100g)	$3.02\pm0.01^{**}$	$6.89\pm0.02^{*}$
Alkaloids (g/100g)	0.03±0.06	0.04±0.02

Values are expressed as mean (n=3) ± SD

Values in the same horizontal row having different superscript differ significantly (P≤0.05).

The result obtained from the phytochemical analysis is depicted in Table 1. The results show that tannin is the major metabolite present in the extract of the samples. However, the tannin content (15.26±0.21g/100g) in the young leaves of this plant differ significantly (P≤0.05) from the content in the extract of the mature leaves. This implies that the amount of tannin synthesize by moringa plants varies considerably in the stage of development of the plant leaves. The older the plant leaves, the higher the tannin content. The values (15.26±0.21 and 25.84±0.61g/100g) of tannin obtained in this study were higher than the values (9.36±0.04 and 9.19±0.02g/100g) previously reported by Nweze and Nwafor (2014). The tannin content obtained in this work can have profound effect on microbial growth and as well affect the bioavailability of some important nutrient if frequently consumed.

The flavonoid contents (3.02±0.01 and 6.89±0.02g/100g) obtained from the analysis of the two samples differ significantly (P≤0.05) Table 1. The role exhibited by flavonoid cannot be left out. Flavonoids can interfere with pathogen growth, probably by enzymes inhibition. The mechanisms of action of flavonoids on microbial growth may be attributed to the inhibition of DNA gyrase, inhibition of Cytoplasmic membrane function and energy metabolism in the micro-organism.

The content of saponin revealed in the methanolic extract of the young leaves is lower than the values (1.46±0.03 and 1.72±0.05g/100g) reported by Nweze and Nwafor, (2014). Such difference may be attributed to the stage of maturity, environmental condition, as well as different solvents used in the extraction.

The alkaloid contents (0.03±0.06 and 0.04±0.002g/100g) present in the two samples revealed no significant difference (P≤0.05). The phytochemical assessment of *Moringa oleifera* leaf by Abalaka *et al.*, (2012) also reveals the presence of alkaloids. Alkaloid has yielded positive result in the treatment of hypertension, diabetes, malaria, cancer, and cough (Akapauaka, 2009).

Results from the antimicrobial effect of the methanolic extract are depicted in (Table II and III). The extracts tested on the growth of *E.coli* revealed zones of inhibition of 26, 21, 17, 13, 8, and 4mm at concentrations of 250, 200, 150, 100, 50 and 25mg/ml (Table II), while the extract from the mature leaves had 34, 23, 20, 14, 8, and 5mm zones of inhibition at various concentrations (Table III). Meanwhile, the control (Amoxicillin) showed 30mm zone of inhibition at 250mg/ml concentration. This implies that the methanolic extract from the mature leaves was more sensitive to the growth of *E.coli*. The results also clearly suggest that the methanolic extract of both samples were effective at higher concentrations. Walter *et al.*, (2011) also revealed that the methanolic extract of *Moringa oleifera* seed was very effective against the growth of *E.coli*. *E.coli* is the common causes of bacterial infections, including Cholecystitis, bacteremia, cholangitis, urinary tract infection and many among others.

The results in Table II and III showed varying inhibition efficacy against *staphylococcus aureus*. The higher zones of inhibition recorded for the methanolic extracts of the young and the mature leaves were 22 and 26mm respectively, while the control had 20mm zone of inhibition. This implies that the two extracts were more efficient than the control. Although the methanolic extract of the mature leaves was more sensitive against the growth of *staphylococcus aureus*. The organism *S.aureus* is responsible for number of diseases and can survive for weeks to months (Cimolai, 2008). Research has also shown that the control of *S.aureus* with oral antibiotics has not been efficacious (Birine *et al*., 2008).

Table II: Antimicrobial activity of the methanolic extract of the young leaves of *Moringa oleifera*.

Microorganisms	250mg/ml	Zone of 200mg/ml	Inhibition 150mg/ml	(mm) 100mg/ml	50mg/ml	25mg/ml	Amoxicillin (control) 250mg/ml
E.coli	26	21	17	13	8	4	30
S.aureus	22	19	18	13	12	7	20
P.aeruginosa	20	19	13	10	9	4	10
***k*.pneumonae**	20	17	8	2	Ns	Ns	10

Ns = Not sensitive

As shown in Table (II and III), the two extracts were more effective against the growth of *pseudomonas aeruginosa* at concentrations of 250, 200, 150mg/ml respectively. Although the mature leaves of this plant showed higher zones of inhibitions (26, 20, 16, 3, 10 and 5mm), while the young leaves showed lower zones of inhibition (20, 19, 13, 10, 9 and 4mm). The control on the other hand revealed the lowest zone of inhibition (10mm) on the test organism (*P.aeruginosa*). Previous report by Akingbade *et al*., (2013) also revealed that the methanolic extract of *moringa oleifera* was sensitive to the growth of *P aeruginosa*. The micro-organism (*P.aeruginosa*) can cause disease in animals, including humans. It may be fetal to the lungs, kidney, urinary tract etc. (Balcht and smith, 1994).

Table III: Antimicrobial effect of the methanolic extract of the mature leaves of *Moringa Oleifera.*

Microorganism	Zone of 250mg/ ml	Inhibition 200mg/ml	(mm) 150mg/ml	100mg/ml	50mg/ml	25mg/ml	Amoxocillin (control) 250mg/ml
E.coli	34	23	20	14	8	5	30
S.aurerus	26	22	20	14	12	10	20
P.aeruginosa	26	20	16	13	10	10	10
K.pneumonae	21	17	10	4	Ns	Ns	10

Ns = Not sensitive

The results in table (II and III) showed that the extracts were not sensitive to the growth of *klebsiella pneumonae* at lower concentrations (50mg/ml and 25mg/ml). However, at concentrations of 250 and 200mg/ml, the plant extracts showed higher zones of inhibition of 20, 17 and 21, 17mm respectively. Meanwhile the control (amoxicillin) had 10mm as the zone of inhibition. This implies that the methanolic extracts of this plant is more effective (at higher Concentrations) against the growth of *K.pneumonae.* The effectiveness of the plant leaves extracts against the growth of *Klebsiella pneumonae* as obtained in this study is more promising than previous report by Makanjuola *et al*., (2013). *Klebsiella pneumonae* can cause destructive changes to human lungs, specifically to the alveoli (in the lungs) resulting in bloody sputum.

CONCLUSION

Conclusively, the results obtained in this study deduced that the older the plant leaves of *Moringa oleifera*, the higher the plant secondary metabolite present, and the better the antimicrobial efficacy of the plant leaves extract.

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