Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 4 - 2021, pag. 371 - 379

THE EVALUATION OF THE ANTICOCCIDIAL PROPERTIES OF AQUEOUS LEAF EXTRACT OF *Moringa Oleifera*

***Anthony Chukwuka MGBOJIKWE[1], Samuel K VIHI[1], Adeola O. OLANIHAN[1], Kingsley I. OKEKE-AGULU[1], Jude O.OKPARA[2]**

[1]*Agricultural Extension and Farm Management Department, Federal College of Forestry, Forestry Research Institute of Nigeria, Jos, Plateau State, Nigeria,* anthonymgbojikwe@fcfjos.edu.ng
[2]*Federal College of Animal Health and Production Technology, National Veterinary Research Institute, Vom, Plateau State, Nigeria*
*Corresponding Author
Received 18th October 2021, accepted 27th December 2021

Abstract: *The purpose of this paper is to evaluate the effect of the aqueous leaf extract of Moringa oleifera extract on Coccidiosis of chicken. Two hundred and forty (240) broiler birds were experimentally infected with Eimeria tenella and treated with the aqueous leaf extract of M. oleifera* at *different concentrations (50mg/ml, 100mg/ml, 200mg/ml and 0%). The birds were divided into four groups T_1, T_2, T_3 and T_4 with three replicates in each group. T_1, T_2, T_3 and T_4 had percentage mortality rate of 16.7%, 8.3%, 5.0% and 91.7%, respectively. Duncan count was carried out at the end of the experiment for the scoring of haemorrhagic lesions. Analysis of variance (ANOVA) showed that there is a significant difference ($p>0.005$) between the different levels of inclusion of aqueous leave extract of M. oleifera* as *was shown in the lesion score. From the study, it is suggested that M. oleifera has great potentials in being used to control coccidiosis in birds given that T_3 had the lowest mortality rate while T_4 (control) had the highest mortality rate. Vitamin and mineral element analysis showed that M. oleifera contains vitamins A, B_1, B_2, B_3, E and C. The minerals M. oleifera contains include calcium, copper, iron, magnesium, phosphorus, potassium and zinc. The phytochemical analysis and thin layer chromatography of the powdered leaf of M. oleifera revealed the presence of alkaloids, yohimbin; tannins, catechin and epicatechin; saponins, β-hederin; glycosides, kaempferol and quercetin; flavonoids, chlorogenic acid, hyperoside, rutosid, rahmnetin and isorahmnetin and terpenoids, oleanoic acid and β-sitosterol, which have all been shown to have retrogressive effect on microorganisms.*

Keywords: *Coccidiosis, Eimeria tenella, Moringa oleifera, Phytochemistry, Thin Layer Chromatography*

1. Introduction

Avian coccidiosis remains one of the most common diseases of poultry industry all over the world. It constitutes an important wet season hazard to poultry farmers in Nigeria [1]. Coccidiosis caused by *Eimeria* species cause huge economic losses in poultry and this includes the costs for treatment of birds, reduced productivity and losses due to mortality of birds [2]. Since the introduction of various anticoccidial drugs and vaccines however, medications with these drugs have been fairly effective in preventing serious outbreaks of the disease; the reason for the emergence of resistant strains of coccidian parasites [3]. Besides the scarcity of livestock drugs [1], access to anticoccidial drugs which is difficult especially to the rural poultry farmers hence the urgent need to develop alternative drugs for the purpose of curing coccidiosis in poultry.

This necessitated the need to look into other ways of controlling this disease notably the use of herbs or plants, such as *M. oleifera* which has been acclaimed both

DOI: https://doi.org/10.4316/fens.2021.039

scientifically [4] and by traditionalists to have curative abilities for several diseases of both man and animals. Therefore, the use of this plant extracts as medicine may alleviates these difficulties, as they are not natural products but may comprise new therapeutic molecules to which resistance has not yet developed [5].

2. Methodology

2.1. Collection of *Moringa oleifera* Leaves

The green leaves of *M. oleifera* was collected from the Eto-baba, Angwan-Rukuba area of Jos, Plateau state. The plant was identified using stock number AH05 in the Herbarium unit of the Forestry Research Institute of Nigeria, Federal College of Forestry, Jos. The leaves were then air dried at room temperature, grinded into powder and stored in a cool dry place.

2.2. Preparation of Plant Extract

The aqueous leaf extract of *M. oleifera* was prepared by dissolving the dried pulverized plant in ethanol at the ratio of 1:3, left to stand for 24hr at room temperature and filtered using Whatman's No. 1 filter paper. The filtrate was then poured into stainless steel plates and dried in hot water bath. The dried extract of *M. oleifera* was then stored in a dessicator.

2.3. Preparation of Test Samples

Three (3) separate test samples were prepared by dissolving the dried extract of *M. oleifera* in distilled water (50mg/ml, 100mg/ml, and 200mg/ml), and stored in a refrigerator at 4^oC for 48 hr.

2.4. Phytochemical Screening of Powdered Plant Parts of *Moringa oleifera*

Phytochemicals such as Alkaloid, Tannin, Glycoside, Resin, Flavonoid and Saponin were analyzed for based on standard acceptable scientific method [6].

2.4.1. Test for Alkaloids

0.5g of the dried pulverized *M. oleifera* was dissolved 5mls of 1% aqueous HCl on a steam bath. This was filtered and 1ml of the filtrate treated with a few drops of Draggendorffs reagent and 1ml of a second portion of the filtrate treated with Wagner's reagent; the formation of precipitates indicated the presence of alkaloid.

2.4.2. Test for Tannins

0.5g of the dried pulverized *M. oleifera* was dissolved in 10ml of distilled water. This was filtered and 2ml of 5% $FeCl_3$ added to the filtrate. A deep green coloration showed the presence of tannins. A second portion of the filtrate was treated with a 2ml of iodine solution. A faint bluish coloration confirmed the presence of tannins.

2.4.3. Test for Saponins

0.5g of the dried pulverized *M. oleifera* was measured, placed into a test tube, 5mls of distilled water added and shaken thoroughly. The formation of froth which persists on warming indicated the presence of saponins.

2.4.4. Test for Flavonoids

0.5g of the dried pulverized *M. oleifera* was measured, placed in a test tube and dissolved in 2mls of dilute NaOH solution. Three drops of concentrated H_2SO_4 were then added. The appearance of a colorless solution indicated the presence of flavonoids.

2.4.5. Test for Resins

0.5g of the dried pulverized *M. oleifera* was measured and placed in a test tube and 5mls of boiling ethanol added. The solution was then filtered using a Whatman's No. 1 filter paper and the filtrate diluted with 4mls of 1% aqueous HCl. The formation of a heavy

resinous precipitate indicated the presence of resins.

2.4.6 Test for Glycosides

0.5g of the dried pulverized *M. oleifera* was dissolved in 10mls of distilled water. The solution was filtered and 2mls of the filtrate hydrolyzed with a few drops of concentrated HCl. The mixture was then alkalized with a few drops Ammonia solution. Five (5) drops of this solution was added to 2mls of Benedict's qualitative reagent and boiled. A reddish-brown precipitate showed the presence of glycosides.

2.4.7 Test for Tannins

A 2g of dried pulverized leaf of *M. oleifera* was treated with Potassium ferric cyanide and ammonia solution. A deep red color indicated the presence of tannins.

2.5. Chromatographic Screening of Phytochemicals in Powdered Plant Parts of *M. oleifera*

The phytochemicals that were qualitatively extracted were then assayed chromatographically using the solvent system ethylacetate-methanol-water in the ratio of 100:13.5:10 [7].

2.5.1. Preparation of Chromatoplates for Thin Layer Chromatographic Analysis

Five (5) chromatoplates were thoroughly washed with water and allowed to dry. The plates were further cleaned with acetone and set on the plate holder. 50g of silica gel powder was dissolved in 110mls of deionized water, the mixture corked and fixed on an Action Wrist Shaker, balanced with 50mls of water and shaken for 30 minutes. The mixture was then applied on the plates and drawn from one to the other with a spreader at a thickness of 0.25mm and allowed to dry for 30 min.

2.5.2. Extraction of Active Principles from Dried Pulverized Leaves of *Moringa oleifera*.

The phytochemicals identified were extracted and prepared for chromatographic screening using a standard acceptable scientific method [7].

I. Alkaloids: 1g of the dried pulverized *M. oleifera* was mixed thoroughly with 1ml of 10% ammonia solution and extracted by shaking for 5 minutes with 5mls of methanol at 60°C on a water bath, filtered and the filtrate used for the chromatography.

II. Saponins: 1g of the dried pulverized *M. oleifera* was shaken for 15 minutes with 20mls of chloroform and filtered. The filtrate was then evaporated to dryness and residue dissolved in 2mls of chloroform/methanol (1:1). The mixture was then used for the chromatography.

III. Flavonoids: 1g of the dried pulverized *M. oleifera* was extracted with 10mls of methanol for 5 minutes on a water bath at 60°C and filtered. The filtrate was used for the chromatographic screening.

IV. Glycosides: 2g of dried pulverized *M. oleifera* leaf was extracted by heating for 15 min under reflux with 30 ml 50% ethanol, with the addition of 10ml 10% lead-(II)-acetate solution. After cooling and filtration, the solution was extracted by shaking with three 15ml quantities of dichloromethane/isopropanol (3:2); shaking must be gentle to avoid emulsion formation. The combined lower phases were filtered over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in 1ml dichloromethane/isopropanol (3:2) and used for chromatographic screening

V. Terpenoids: 1g of dried pulverized *M. oleifera* leaf was extracted for 15 min with 15ml methanol under reflux. The filtrate was evaporated to 3ml and 30µl was used for the chromatographic screening.

VI. Tannins: Samples were prepared by diluting the crude extracts of chloroform, acetone, methanol and water with 1g of dried pulverized *M. oleifera* leaf and then applied using 1-10µl volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes.

2.5.3. Activation of Chromatoplates

The chromatoplates were set in the hot air oven and activated at 110°C for 30 minutes, removed and allowed to cool to room temperature.

2.5.4. Application of Extracts on Chromatoplates.

Each of the extracts were spotted on the chromatoplates on a line from the lower edge (origin) of the plates with a spacing of 2-3cm in-between.

2.5.5. Saturation of the Chromatographic Tank

The chromatographic tank was saturated for 1 hour with the developing solvent chroloform/ methanol (85:15) for alkaloids; chloroform/methanol/water (64:50:10) for saponins; n-butanol/glacia-acetic acid/water (40:10:50) for flavonoids and chloroform/methanol (95:5) for the tannins, respectively. The spotted plate was then placed in the chromatographic tank containing the solvent after petroleum jelly (Vaseline) had been applied on its edges and the lid covered. When the solvents reached the score line or solvent front, the plate was removed and reactivated in an oven at a temperature of 110°C for 10 minutes.

2.5.6. Visualization and Identification of Separated Components

The chromatographic film was set in the ultra violet light machine and viewed under the short wave length. The individual components were identified by their characteristic colors and their R_f values. The R_f values obtained were compared with those of known standard in a standard atlas.

$$R_f = \frac{\text{Distant moved by the solute}}{\text{Distant moved by the solvent}}$$

2.6. Vitamin and Mineral Element Analysis

Various vitamins and some macro and micro elements were determined after ashing and digestion, using Atomic Absorption Spectrometer (Model DW-AA4530F) according to the method described by the Association of the Official Analytical Chemist [8].

2.6.1. Ashing and Digestion

A 2g of the dried pulverized *M. oleifera* leaves were weighed into a crucible and ashed in a murfle furnace preheated to 600°C for 4 hours. The crucible was then transferred directly to a desicator and allowed to cool. The various ash from different parts of the plant were then separated and treated with a few millilitres of HCl, few drops of concentrated HNO_3 and boiled. These were then cooled and filtered. The filtrate made up to 10mls in a standard volumetric flask with deionized water. These solutions were used for the determination of cations.

2.6.2. Determination of Cations

Seven (7) cations; sodium, calcium, magnesium, potassium, phosphorus, selenium and lead were determined with the aid of Atomic Absorption Spectrometer. The results were converted from ppm to mg per 100g using the formular:

$$mg/100g = \frac{conc.(ppm)}{10} \times \frac{Soln.Vol}{wt.(g)^2} \times \frac{100}{1}$$

2.7. Experimental Organisms

The experimental organism (*Eimeria tenella*) was sourced from the Parasitology Division of the National Veterinary

Research Institute Vom, Plateau State Nigeria.

2.8Experimental Birds

Two hundred- and forty-day-old broilers chicks were brooded for three weeks within which routine management practices were observed. At three (3) weeks of age, the birds were divided into four (4) groups (T_1, T_2, T_3 and T_4) with three (3) replicates in each group.

2.8.1. Experimental Infection with *Eimeria tenella* and Treatment with the Plant Extract.

Challenge of birds with *E. tenella* was carried out at 30 days of age for two consecutive days and on observation of clinical signs, treatment with the plant extract commenced. To groups T_1, T_2 and T_3 were administered 50mg/ml, 100mg/ml and 200mg/ml of the aqueous leaf extract of *Moringa oleifera* for five (5) days, respectively. To group T_4, no treatment was administered (control).

2.8.2. Performance Parameters.

The birds were observed daily for clinical signs. Morbidity and mortality were recorded daily in each sub-group. Necropsies were carried out on the birds that died during the experiment.

Seven days after the challenge, the evaluation of caecal lesions was carried out on fifteen birds from each group. A lesion score was assigned from 0 to 4, where 0 corresponds to normal status with no gross lesion, 1 to small scattered petechiae, 2 to numerous petechiae, 3 to extensive petechiae and 4 to extensive haemorrhage that gives a dark color to the caecal intestine [9].

2.9 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using statistical package for social science (SPSSLTD, working, surrey, UK). The significant treatment effect was discussed at probability significant means and was separated using the Duncan's new multiple range test and test was considered significant at a probability of $p<0.05$.

3. Result and Discussion

M. oliefera has been reported to have a strong antioxidant effect against prostrated band skin cancers, an anti-tumor and an anti-aging substance [10]. Furthermore, *M. oliefera* modulates anaemia, high blood pressure, liver and kidney problems. *M. oliefera* has strong anti-inflammatory properties and ameliorating rheumatism, joint pain, arthritis and lupus. The plant is effective against digestive disorders including colitis, diarrhea, and flatulence (gas), ulcer or gastritis. *M. oliefera* is an anti-bacterial and anti-viral agent; it is effective against urinary tract infection, detoxifer and outstanding immune builder [11]. It is used in many countries to treat malnutrition and malaria [11]. *Moringa oleifera* is widely regarded by water purification experts as one of the best hopes for reducing the incidence of the water borne diseases. *M. oleifera* is claimed to be the most nutrient-rich plant yet discovered [12].

3.1. Phytochemical Screening

The dried powdered leaves of *M. oliefera* was subjected to phytochemical screening for various phytochemicals according to the method [6]. *M. oliefera* leaf contains tannins, saponins, flavonoids, glycoside and alkaloids highly present, and terpenoids moderately present (Table 1). Tannins is used in styptic preparations which produce contractions of blood vessels; stopping bleeding having the quality of retaining hemorrhages when applied to the bleeding part. Saponins have the property of causing haemolysis of cells even at low dilution, tends to be deposited

on the surface of cells with which they come in contact and are not absorbed by the normal epithelium of the alimentary canal. The presence of saponin in the plant is a demonstration of the fact that the plant may have expectorant actions which are very useful in the management of inflammation of the upper respiratory tract in addition to its cardio-tonic properties as reported by [13] and [6]. Alkaloid which represents the active principle of vegetable drugs, are alkaline in reaction and richly combine with acids, forming salts soluble in water. Some drugs may contain more than alkaloid and the actions of these may be antagonistic to each other. Alkaloid produces analgesic, anti- inflammatory and adaptogenic effects which help to develop resistance against disease and endurance against stress [14].

Glycosides have a tendency to block the conduction of the electrical impulse that causes contraction as it passes from the atria to the ventricles of the heart. Cardiac gylcosides also have a tendency to produce an abnormal cardiac rhythm by causing electrical impulses to be generated at points in the heart other than the normal pace marker region, the cells that rhythmically maintain the heartbeat [15].

Flavonoids are used as a supplement which reduces the symptoms of hemorrhoids. A number of flavonoids have been shown to have anti-inflammatory effects and to strengthen blood vessels. Flavonoids have been investigated for possible anti-inflammatory effects and anti-viral properties. Chlorogenic acid is one of the most important flavonoids in the field of pharmacology- has been shown to have antibacterial, antimutagenic, antitumor, and antiviral activities, plus antioxidant and clastogenic activities. The transisomer acts as an insect oviposition stimulant, and it may also reduce larval growth [16].

Diverse functional roles of terpenoids have been critically studied and well-accepted now. Some of them include natural flavor additives for food or fragrances in perfumery and in traditional and alternate medicines used in aromatherapy. Terpenoids have been extensively studied for its effect in the prevention and treatment of cancer. Illustratively, Taxol derivative (paclitaxel and docetaxel) are among the widely used drugs in cancer chemotherapy. Other important therapeutic uses of terpenoids include antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants, antiparasitic, immunomodulatory, and as skin permeation enhancer [17].

Table 1
Phytochemicals found in *M. oliefera* Leaves.

Phytochemicals	Concentration
Alkaloids	+++
Saponins	+++
Tannins	+++
Terpenoids	++
Glycosides	+++
Flavonoids	+++

KEY: = - Negative
+ = Mildly present
++ = Moderately present
+++ = Highly present

3.2. Chromatographic Screening of *M. oleifera*

Result after the chromatographic screening of the leaves of *M. oleifera* showed Alkaloid, Yohimbin with Rf value 0.80; Saponin, β-hederin with Rf value 0.80; Flavonoids, chlorogenic acid, hyperoside, rutosid, rahmnetin and isorahmnetin with Rf values 0.75, 0.60, 0.39, 0.64 and 0.09, respectively; glycoside (cardiac) kaempferol and quercetin with Rf values 0.12 and 0.63, respectively; Tannin catechin and epicatechin with Rf value 0.20 and 0.36, respectively; terpenoids oleanic acid and β-sitosterol with Rf values 0.39 and 0.52, respectively; saponin β-hederin

with Rf value0.80. The chromatogram did not indicate any spot for Bitter principles (Table 2).

Table 2
Chromatogram of Active Principles Present in *M. oleifera Leaf*

Chemical Group	Rf Value	Possible Identity
Alkaloids	0.80	Yohimbin
Saponins	0.80	β-hederin
Tannins	0.20	Catechin
	0.36	Epicatechin
Terpenoids	0.39	Oleanoic acid
	0.52	β-sitosterol
Glycosides	0.12	Kaempferol
	0.63	Quercetin
Flavonoids	0.75	Chlorogenic acid
	0.60	Hyperoside
	0.39	Rutosid
	0.64	Rahmnetin
	0.09	Isorahmnetin

3.3. Vitamin and Mineral Element Analysis of *M. oleifera* leaf

Water soluble vitamins such as vitamins A, B_1, B_2, B_3 and C were observed in both the fresh and dried leaves of *M. oleifera*. Fat soluble vitamins observed include, vitamins A and E (Table 3). The minerals *M. oleifera* contains include Calcium, Copper, Iron, Magnesium, Phosphorus, Potassium and Zinc. It was observed that the concentrations of the vitamins were exponentially increased in the dried *M. oleifera* leaf which may be as a result of the loss of moisture content in the dried leaves. This result agrees with the result of [12], howbeit with variations in some of the figures recorded. These variations may be as a result of differences in the locations where the plants were harvested. [18] reported that the chemical composition of a plant species is affected by geographical location; factors such as soil type, soil nutritional content and climate have been implicated in altering the concentrations of chemicals found in plants.

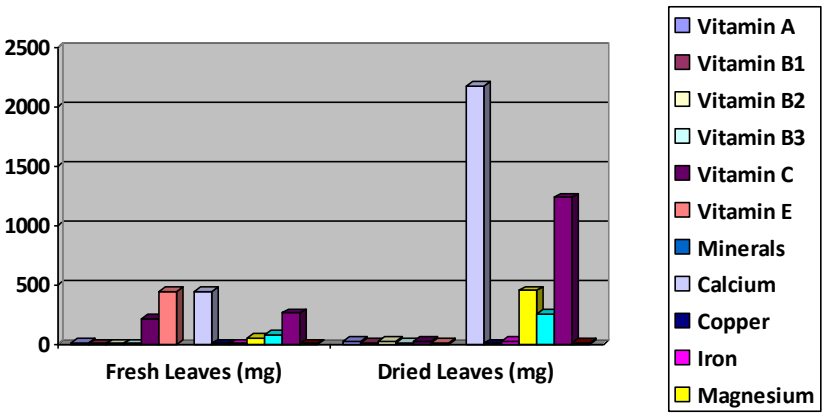

Fig. 1: Vitamins and Minerals Present in *M. oleifera* leaf

3.4. Statistical Analysis

From table 4, the null hypothesis was rejected and accepts alternative hypothesis (Ha) because the figure calculated is greater than the figure tabled from the ANOVA result. This indicates that there is a significant difference between the different levels of inclusion of aqueous leave extract of *M. oleifera* used in the experiment.

Table 4
Analysis of Variance (ANOVA) of the Degree of Hemorrhage of Birds, Experimentally infected with *Emieria tenella*

SOV	SS	DF	MS	F-RATIO	F-TAB
Total	29.3	11	2.66		
Treatment	29.24	3	9.75	1300	4.066
Error	0.06	8	0.0075		

Source: field survey, 2018. Fcal>Ftab

3.5. Performance of Birds infected with *Eimeria tenella* and treated with*Moringa oleifera*

Table5 shows the mortality rate in percentage of birds infected with *Eimeria tenella*. In treatment 4 (T_4) which is the control, had the highest mortality of 91.7% while treatment 1 (T_1) and 2 (T_2) has 16.7% and 8.3%, respectively while treatment 3 (T_3) had 5.0%; the lowest mortality rate. The result shows that the birds that were treated with 200mg/ml of the aqueous leaf extract of *M. oleifera* responded more positively to the treatment compared to other levels of concentration. Mortality result showed that a farm can have a 100% mortality rate when there is a coccidiosis outbreak.

Table 5
Percentage Mortality of Birds Experimentally Infected with *Eimeria tenella* and Treated with Different Concentrations of aqueous Leaf extract of *M. oleifera.*

Treatments	Number of Birds	Mortality	Percentage (%) Mortality
T1 (50mg/ml)	60	10	16.7
T2 (100mg/ml)	60	5	8.3
T3 (200mg/ml)	60	3	5.0
Control (0mg/ml)	60	55	91.7

Source: field survey, 2018.

4. Conclusion

From the experiment, it has been shown that *M. oliefera* has great potentials being used in the treatment and control of cocciodiosis of birds. The phytochemicals identified has been shown to have a wide range of health benefits which amongst others include antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism and antiviral/ antibacterial effect. These phytochemicals have also been shown to help in slowing the aging process and reduce the risk of many disease [19].

The *M. oleifera* plant is the most inexpensive and credible alternative to providing good nutrition. *M. oleifera* is the most nutrient-rich plant yet discovered. *M. oleifera* is an extraordinary plant because all parts of the tree are edible, but the most amazing aspect is its exceptionally high nutritional value. *M. oliefera* provides a rich and rare combination of nutrients, amino acids, antioxidants, anti-aging and -inflammatory properties used for nutrition and healing. The leaves of the *M. oliefera* tree are an excellent source of vitamin A (four times the amount in carrots), the raw leaves are rich in vitamin C (seven times the amount in oranges), and they are also a good source of vitamin B and other minerals. The leaves are also an outstanding source of calcium (four times the amount in milk), protein (twice the amount in milk), and potassium (three time the amount in bananas). The content of iron is very good as well and the leaves have purportedly been used for treating anemia in the Philippines. The content of amino acids such as methionine and cystine is also high. Carbohydrates, fats and phosphorous content are low making this one of the finest plant foods to found.

5. Acknowledgements

- The Herbarium unit, federal College of forestry, Jos Plateau state, Nigeria.
- The Parasitology Division, National Veterinary Research Institute, Vom, Nigeria.
- Biochemistry Division, National Veterinary Research Institute, Vom, Nigeria.

6. References

[1] CHAH, K.F. AND N.E. NWEZE 2001): Antibiotics use in poultry production in Nsukka, southeastern Nigeria, and Prod. 25: 303-306.

[2] UMAYA, R. S. AND PARVATHAM, R. (2012). *Protective efficacy of Moringa oleifera during aflatoxin exposure in broiler.*Res. J Biotech, 2012; 7.

[3] HELMUTH, R. AND D. PROTZ. (2004). How to modify conditions limiting resistance in bacteria in animal and other reserviours.Clin. Infect. and Dis. 24 (Suppl.): pp.136-139

[4] OLA-FADUNSINS, D. AND ADEMOLA I.O. (2013). *Direct effect of Moringa oleifera Lam (Moringaceae) acetone leaf extract on broiler chickens naturally infected with Eimeria species.* Trop Anim Health prod, 2013; 45:1423-1428.

[5] ADAMU, M., AND CHAIWAT B. (2013): *Protective Effects of Moringa stenopetala leaf supplemented Diets on Eimeria tenella infected Broiler Chickens in Dedre Zeit, Central, Ethiopia.*Kasetstart J. (Nat. Sci.) 47: 398-406.

[6] TREASE, G. E. AND EVAN, W.C. (2000). In: A textbook of Pharmacognosy, 13th edition, Bailiere Tindall Ltd; London. Pp 795.

[7] WAGNER, H., BLADT, S. AND ZGAINSKI, E. M. (1984). In: Plant Drug Analysis. A Thin Layer Chromatography Atlas. Berlin Heidelberg Springer-Verlag. 319 pp

[8] AOAC (1975): Official Methods of Analysis. Association of official analytical Chemists. Washington, D.C, USA, PP 450

[9] JOHNSON J. AND REID W. M. (2001). Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chicken, Exp. Parasitol. 28 (1970) 99-102.

[10] OSILA, K. (2012). Harnessing the potentials Of *Moringa*Tree.Posted in The Tide news online, Feb 8th, 2012.

[11] DAHOT, M. U. (2003).*Antimicrobal Activity of Small Protien of Moringa oleifera leaves.* J. Islam. Acad. Sci., 11:1, 27-32.

[12] RAM, C. D., SHEO, D. M., BRIJENDRA, K. P., NARENDRA, B. AND MANISHA, G. (2011). Moringa: The herbal gold to combat malnutrition. Chronicles of Young Scientists. Vol. 2. Issue 3.
https://www.researchgate.net/publication/216463190_Moringa_The_herbal_gold_to_combat_malnutrition

[13] FRANTISEK, S. S. (2002): the natural guide to medicinal herbs and plants. Tiger Barks Institute Twickenham, U.K. Pp. 21-70.

[14] GUPTA, S.S. (2007). Prospects and prospective of natural products in Medicine Indian Journal of Pharmacology, 26:1-12

[15] CLARK A.M. (2009). National products as resources for new drugs, Pharm. Res., 3, 1133-11411

[16] JEFFERY, B. H. AND HERBERT, B. (1993). Phytochemical Dictionary- A Hand book of Bioactive Compounds from plants. Taylor and Francis Limited Pp. 475.

[17] PRIYANKA, P. B. AND PATHIK S. B. (2013). Terpenes: Chemistry, Biological Role, and Therapeutic Applications. Natural Products pp 2665-2691.

[18] MGBOJIKWE, L. O. (2000). Acaricidal Properties of the Aqueous Stem Bark Extract of *Adenium Obesum.* Pp.151-152.

[19] PALOMBO, E. A. (2006). *Phytochemicals from Traditional Medicine plants used in the treatment of Diarrhoea: Mode of action and effects on intestinal function.*Phyother Research. 2006 Sep, 20 (9): 717–24.