Preliminary Investigation on the Chemical Composition of Local Medicinal Herbs (*Curcuma longa* L., *Persicaria odorata* L. and *Eleutherine palmifolia* L.) as Potential Layer Feed Additives for the Production of Healthy Eggs

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ABSTRACT This study was carried out to determine the phytochemical and proximate compositions of the three selected local medicinal herbs: *Curcuma longa* L., *Persicaria odorata* L. and *Eleutherine palmifolia* L. as potential layer feed additive for the production of healthy eggs. In spite of easy cultivation and availability, these three herbs were selected as they are quite common as basic cooking ingredients and food additives in local cuisine. Fresh *Curcuma longa* L. (rhizome), *Persicaria odorata* L. (leaves) and *Eleutherine palmifolia* L. (bulb) were obtained from local markets at Ranau and Sandakan (Sabah). Fresh samples were washed and dried completely in oven (±55°) before being pulverized into powder form and kept in sealed polythene bags under room temperature prior the analysis of proximate and phytochemical analysis. Values obtained from the proximate analysis of the Curcuma longa L., Persicaria odorata L. and Eleutherine palmifolia L. were shown as following; moisture (9.13%, 88.64% and 84.08%), ash (2.07, 1.83 and 2.16%), crude protein (6.19%, 3.5% and 7.62%), crude fat (5.08%, 0.83% and 0.24%), crude fibre (5.85%, 10.66% and 2.68%), Calcium (0.2%, 0.47% and 0.06%) and Phosphorus (0.43%, 0.12% and 0.14%) respectively; Meanwhile, the results of phytochemical screening test revealed that there were presence of saponins, tannins, total phenols, flavonoids and alkaloids in the three herbs as plant secondary metabolites. Results of this study suggest some merit on the chemical compositions of these three herbs which could be used as references in the further study of this project.

KEYWORDS: Local medicinal herbs, Curcuma longa L., Persicaria odorata L., Eleutherine palmifolia L. I Received 2 May 2019 II Revised 27 June 2019 II Accepted 6 August 2019 II Online 28 August 2019 II © Transactions on Science and Technology I Full Article

INTRODUCTION

Plants have known to be the most prominent elements in the ecosystem as they contribute fundamental basis for all life on earth. Being part of food sources, plants in general provide adequate sources of essential nutrients such as fibre, mineral and vitamins that required in our daily diets. Besides, herbal plants have also been used as folk medicines as natural therapeutic agents and medicaments before the development of modern medicines since thousand years ago to treat various illness, infections and other health disorders until today. It is found that these plants in nature exhibit broad spectrum of biological and pharmacological properties which are good in promoting health due to the actions of primary and secondary metabolites in plants which have multiple biological characteristics such as anti-inflammatory, anti-bacterial and anti-oxidant effects, in other words, single drug may be simultaneously use to treat different diseases (Wink, 2015; Rodriguez-Garcia *et al.*, 2017). Unlike modern medicines such as antibiotics, it is demonstrated that these medicinal herbs are naturally available and safe as they do not produce any side effects, such

as drug resistance (Padhi *et al.*, 2015). In recent reports, WHO revealed that nearly 60% of the world's population still relies on traditional natural herbal medicines for primary health care needs, for examples as recorded in traditional Chinese, Ayurverdic and Kampo medicinal system (Usman *et al.*, 2009). Thus, researches on plant-derived drugs have been continuously carried out to screen more unexplored plants around the globe for documentation as well as search for new potential drugs from natural sources as the alternative to modern medicines. As in human health, same trends have also been observed in animal especially livestock sector, the demand for such approach is growing with the concerns of healthiness and organic lifestyle practices. With the banning of antibiotic growth promoters by European in livestock sector since 2006, researches and feeding trials have been conducted by including certain level of these plant-based compounds such as garlic and oregano in animal diets with the aims of improving the animal production performances and their products quality without the use of antibiotic cum growth promoters.

Curcuma longa L., or turmeric, is a perennial herb that originated from India which then widely found in tropical and subtropical regions (Beevers & Huang, 2011; Rahardja *et al.*, 2015). In the olden days before the ancient populations discovered its medicinal values, it is cultivated to use as coloring and flavoring agent besides being used in both Hindu and Buddhist religious ceremonies due to the bright yellow pigments given by its main active compounds – curcuminoids (Nair, 2013). It is revealed that these active compounds tend to exhibit antioxidant, antimicrobial, hepatoprotective and other properties, even act as potent bioprotectant as well, enabling it to be used in treating various illness such as gastrointestinal and respiratory disorders (Nair, 2013; Labban, 2014; Kafi *et al.*, 2017). In researches regarding animal nutrition, curcumin in turmeric is reported to aid in improving the liver function which in turn increased the synthesis of vitellogenin and follicular development, thus increased the egg production performance (Saraswati *et al.*, 2013). This can be explained due to the potent antimicrobial activity of the herbal plant that improved the intestinal environment by suppressing the colonization of pathogens, thus resulting in better nutrient digestion and absorption in the gut (Ooi *et al.*, 2018).

Persicaria odorata L., or Vietnamese coriander, or in Malaysia named as 'Daun laksa', is an indigenous perennial herb from Southeast Asia (Dash *et al.*, 2016). It is often used in local cuisine of Vietnam, Malaysia and Singapore such as Laksa soup as it has strong lemony and mint-mimic scent which gives the dishes a unique taste (Sasongko *et al.*, 2011a). This unique pungent smell is reported due to the presence of (Z)-3-hexenal, (Z)-3-hexen-1-ol, decanal, undecanal, and dodecanal (Starkenmann *et al.*, 2006). According to traditional healing practices, the leaves of *Persicaria odorata* was used as folk medicine to treat various ailments such as diarrhoea and inflammation as it encompasses varied biological properties including antibacterial and antifungal, antioxidant, antidiabetic and others which are probably due to the presence of major volatile compounds such as long-chain aldehydes (eupatoriochromene, dodecanal and others) and other plant secondary metabolites (flavonoids and phenolic compounds) (Dash *et al.*, 2016; Nanasombat *et al.*, 2009; Sasongko *et al.*, 2011b; Ridzuan *et al.*, 2013).

Eleutherine palmifolia (L.) Merr, or Dayak onion is an indigenous herbaceous plant that originated from South America and widely grown in the regions of Africa, Malaysia, Indonesia and Philippines (Agustin *et al.*, 2016). The main part of *Eleutherine palmifolia* is its bulb which often freshly used in culinary or in the form of dried onion bulb, pickles, or even powder form. Recent researches revealed that bio-active compounds such as naptoquinones (elecanacine, eleutherine, eleutherol, eleutherinone) associated with the secondary metabolites in the bulb, tend to give a wide range of vital biological effects including antioxidant, antimicrobial, antimitotic, immunostimulant and others (Agustin *et al.*, 2016; Harlita *et al.*, 2018). Therefore, it is popular among Dayak tribe in Kalimantan as

natural multi-function medicine to treat illness such as high blood pressure, diabetes, high cholesterol and stroke, as well as for postpartum healing process (Kuntorini *et al.*, 2010; Harlita *et al.*, 2018). Moreover, it is found that ethanol extract from the bulb could inhibit the growth of pathogenic bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Shigella* sp., *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli* (Harlita *et al.*, 2018).

It is found that there is still lacking of information regarding the inclusion of both *Persicaria* odorata L. and *Eleutherine palmifolia* L. in animal nutrition, therefore, this study aimed to present a preliminary investigation on the chemical composition of these three local medicinal herbs (*Curcuma longa* L., *Persicaria odorata* L. and *Eleutherine palmifolia* L.) as an advance to introduce as potential layer feed additive for the production of healthy eggs as well as to improve layer production performance.

METHODOLOGY

Preparation of Plant Materials

Fresh *Curcuma longa* L. (rhizome), *Persicaria odorata* L. (leaves) and *Eleutherine palmifolia* L. (bulb) were obtained from local markets at Ranau and Sandakan, Sabah. The fresh samples were washed and dried completely in oven (±55°) before being pulverized into powder form and stored in sealed polythene bags under room temperature.

Determination of Proximate Composition

Dry matter, moisture, ash, crude protein, crude fat and crude fibre contents were determined as according to the methods of analysis of the Association of Official Analytical Chemists (AOAC, 2000); meanwhile calcium and phosphorus were determined spectrophotometrically as described in AOAC (1990). All analyses were carried out in triplicate and results obtained were reported in term of percentages.

Determination	of Phi	ytochemical	(qualitative	<i>screening test</i>)
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Table 1. Qualitative screening methods for the determination of phytochemical.

Saponins	Frothing test: 5 ml of extract was added with 20 ml of distilled water a			
	followed by agitation for 15 minutes. Formation of foam indicated its			
	presence.			
Tannins	Acid test: 5 ml of extract was added with 2 ml of 1% hydrochloric acid.			
	Deposition of red precipitate indicated its presence.			
Total phenols	Ferric Chloride test: 1 ml of extract was added with 1 ml of 10% ferric			
	chloride. Formation of greenish-brown or black precipitate or colour was			
	taken as positive result for its presence.			
Flavanoids	Sodium hydroxide test: Few drops of diluted sodium hydroxide was			
	added into 1 ml of extract. Formation of precipitate indicated its presence.			
Alkaloids	Wagner's test: 1 ml of extract was shaken with 5 ml of 2% hydrochloric			
	acid on a steam bath and filtered. Wagner's reagent (iodine in potassium			
	iodide solution) was then added and observed. Formation of reddish-			
	brown precipitate indicated its presence.			

RESULT AND DISCUSSION

Table 2 shows the results of chemical composition and qualitative phytochemical screening of Curcuma longa L., Persicaria odorata L. and Eleutherine palmifolia L. From the table, it is revealed that Curcuma longa L., Persicaria odorata L. and Eleutherine palmifolia L. contain desirable amounts of moisture (9.13%, 88.64% and 84.08%), ash (2.07, 1.83 and 2.16%), crude protein (6.19%, 3.5% and 7.62%), crude fat (5.08%, 0.83% and 0.24%), crude fibre (5.85%, 10.66% and 2.68%), Calcium (0.2%, 0.47% and 0.06%) and Phosphorus (0.43%, 0.12% and 0.14%) respectively. For the proximate composition, the results of Curcuma longa L. in this study were set as references to compare with the results of Persicaria odorata L. and Eleutherine palmfolia L. as both of them have limited findings on this aspect. The result of *Curcuma longa* L. in present study on the proximate composition is nearly in line with the results as reported by Ikpeama et al., (2014) and Imoru et al., (2018), except for crude protein content they obtained slightly higher values ranging between 9.40 - 10.07%. However, Nair (2013) summarized the crude protein content in turmeric is 6.3%. In 2018, Imoru et al. demonstrated that the presence of these essential nutrients in *Curcuma longa* L. could eventually help to enhance overall production performance of poultry, in which constant feeding at appropriate amount could bring health benefits such as bone and skeletal development and energy metabolism (Latunde-dada, 1990; Kubmarawa et al., 2007; Ikpeama et al., 2014 and Ooi et al., 2018).

Meanwhile, the phytochemical screening test of the three herbs indicated there were presence of saponins, tannins, total phenols, flavonoids and alkaloids. These phytochemicals, also known as plant secondary metabolites or non-nutrient plant compounds, are reported to have pharmacologically active agents that give protective effect against diseases (Soetan and Oyewole, 2009). Researches revealed that different phytochemicals were known to play important roles in antimicrobial, curative and other vital physiological activities against several pathogenic bacteria, and thus allowing them to be used as a cheap source of herbal drugs to treat various illnesses (Hassan et al., 2004; Usman and Osuji, 2007; Usman et al., 2009). Hence, the medicinal properties of these herbs can be partly credited to the presence of these phytochemicals. For example, both saponins and tannins are reported to be active antifungal and antimicrobial agents which could inhibit the growth of microorganisms by precipitating microbial protein that are required for the survival of microorganisms, thus explained their uses in treating intestinal disorders (Dharmananda, 2003; Prasad et al., 2008; Ikpeama et al., 2014). In 2018, Ooi et al. (2018) demonstrated that supplemented 1% of Curcuma longa L., Persicaria odorata L. and Eleutherine palmifolia L. respectively in layer feed helped to improve the faecal microbial bacteria counts, indicating the improvement of intestinal environment as these phytochemicals were reported to have selective inhibition between beneficial intestine microflora and pathogenic bacteria (Sharma et al., 2006; Cardelle-cobas et al., 2010). Besides, researches also revealed that saponins possess ranges of health-beneficial properties such as hepato-protective, hypoglycemic, hypocholesterolemic and antidiabetic (Rupasinghe et al., 2003; Addisu et al., 2016). Furthermore, flavonoids and phenols has also been demonstrated as effective biological radical scavenger, making it to be potent antioxidant which allowing it to exhibit wide range of health-promoting activities such as anti-inflammatory, antiangionic, antioxidant and other properties (Gill, 1992; Ikpeama *et al.*, 2014). According to Imoru *et al.* (2018), optimum level of these phytochemicals in diet could possess positive effects on of both human and livestock health.

Parameters	Curcuma longa L.	Persicaria odorata L.	Eleutherine palmifolia L.
Proximate composition (%)			
Moisture	9.13 ± 0.07°	88.64 ± 0.06^{a}	84.08 ± 0.09^{b}
Ash	2.07 ± 0.03^{b}	$1.83 \pm 0.02^{\circ}$	2.16 ± 0.02^{a}
Crude protein	$6.19\pm0.10^{\mathrm{b}}$	$3.50 \pm 0.07^{\circ}$	7.62 ± 0.07^{a}
Crude fat	5.08 ± 0.07^{a}	0.83 ± 0.04^{b}	$0.24 \pm 0.04^{\circ}$
Crude fibre	5.85 ± 0.05^{b}	10.66 ± 0.08^{a}	$2.68 \pm 0.06^{\circ}$
Calcium	0.20 ± 0.02^{b}	0.47 ± 0.01^{a}	$0.06 \pm 0.01^{\circ}$
Phosphorus	0.43 ± 0.02^{a}	0.12 ± 0.01^{b}	0.14 ± 0.02^{b}
Qualitative phytochemical	screening		
Saponins	+	+	+
(Frothing test)			
Tannins	+	+	+
(Acid test)			
Total phenols	+	+	+
(Ferric chloride test)			
Flavonoids	+	+	+
(Sodium hydroxide test)			
Alkaloids	+	+	+
(Wagner's test)			

Table 2. Chemical compositions and qualitative phytochemical screening of *Curcuma longa* L., *Persicaria odorata* L. and *Eleutherine palmifolia* L.

¹ a-c Mean values ± SE with different letters in the same row are significantly different (P<0.05).

² '+' indicated the presence of component.

CONCLUSION

Results obtained from present study indicated that these three herbs (*Curcuma longa* L., *Persicaria odorata* L. and *Eleutherine palmifolia* L.) contain desirable amount of nutrients. The presence of phytochemicals (saponins, tannins, total phenols, flavonoids and alkaloids) in these three herbs indicated their potential medicinal effects, along with various vital biological and physiological properties, which could use to improve both human and animal health. Lastly, results of present study might be used as references in the further study of this project by supplementing the three herbs in layer diet as feed additive to improve production performance and produce healthy eggs.

ACKNOWLEDGEMENTS

This research was funded by Universiti Malaysia Sabah (SDK-009) and authors were grateful to Faculty of Sustainable Agriculture, Universiti Malaysia Sabah for providing support and facilities.

REFERENCES

- [1] Addisu, S., & Assefa, A. (2016). Role of Plant Containing Saponin on Livestock Production; A Review. *Advances in Biological Research*, **10**(5), 309-314.
- [2] Agustin, A. R., Faika, S. & Ju, Y. H. (2016). Influence of Extracting Solvents on its Antioxidant Properties of Bawang Dayak (*Eleutherine palmifolia* L. Merr). *International Journal of Chemical and Petrochemical Technology*, **6**(2), 1-10.

- [3] AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists (15th edition). Washington DC: AOAC International Publisher.
- [4] AOAC. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists (17th edition). Washington DC: AOAC International Publisher.
- [5] Azwanida, N. N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Journal of Medicinal and Aromatic Plants*, **4**(196), 196.
- [6] Beevers, C. S. & S. Huang. (2011). Pharmacological and Clinical Properties of Curcumin, Review. *Botanics: Targets and Therapy*, **1**(5), 5-18.
- [7] Cardelle-Cobas, A., Soria, A. C., Corzo-Martinez, M. & Villamiel, M. (2010). A Comprehensive Survey of Garlic Functionality. *Garlic Consumption and Health*, **1**, 1-60.
- [8] Dash, G. K. & Zakaria, B. (2016). Pharmacognostic Studies on *Persicaria odorata* (Lour.) Sojak. *Journal of Pharmacy Research*, **10**(6), 377-380.
- [9] Dharmananda, S. (2003). Gallnut and The Uses of Tannins in Chinese Medicine. *Proceedings of institute for traditional medicine*. September, 2003. Portland, Oregon.
- [10] Gills, L. S. (1992). Ethno Medical Uses of Plants in Nigeria. Benin: African press.
- [11] Harbone, J. B. (1998). *Methods of Extraction and Isolation 'Phytochemical Methods'* (3rd edition). London: Chapman and Hall Ltd.
- [12] Harlita, T. D., Oedjijono & Asnani, A. (2018). The Antibacterial Activity of Dayak Onion (*Eleutherine palmifolia* L. Merr) towards Pathogenic Bacteria. *Tropical Life Sciences Research*, 29(2), 39-52.
- [13] Hassan, M. M., Oyewale, A. O., Amupitan, J. O., Abduallahi, M. S. & Okonkwo, E. M. (2004). Preliminary Phytochemical and Antibacterial Investigation of Crude Extracts of the Root Bark of Detarium microcarpum. Journal of Chemical Society of Nigeria, 29(1), 26-29.
- [14] Ikpeama, A., Onwuka, G. I. & Nwankwo, C. (2014). Nutritional Composition of Turmeric (*Curcuma longa*) and Its Antimicrobial Properties. *International Journal of Scientific and Engineering Research*, 5(10), 1085-1089.
- [15] Imoru, A., Onibi, G. E. & Osho, I. B. (2018). Nutritional and Biochemical Compositions of Turmeric (*Curcuma longa Linn*) Rhizome Powder – A Promising Animal Feed Additive. International Journal of Scientific & Engineering Research, 9(1), 424-429.
- [16] Kafi, A., Uddin, M. N., Uddin, M. J., Khan, M. M. H. & Haque, M. E. (2017). Effect of Dietary Supplementation of Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) and Their Combination as Feed Additives on Feed Intake, Growth Performance and Economics of Broiler. *International Journal of Poultry Science*, 16, 257-265.
- [17] Kubmarawa, D., Ajoku, G. A., Enwerem, N. M. & Okorie, D. A. (2007). Preliminary Phytochemical and Antimicrobial Screening of 50 Medicinal Plants from Nigeria. *African Journal of Biotechnology*, 6(14), 1690-1696.
- [18] Kuntorini, E. M & Nugroho L. H. (2010). Structural Development and Bioactive Content of Red Bulb Plant (*Eleutherine Americana* Merr.): A Traditional Medicine for Local Kalimantan People. *Biodiversitas*, 11(2), 102-106.
- [19] Labban, L. (2014). Medicinal and Pharmacological Properties of Turmeric (*Curcuma longa*): A review. *Journal of Pharmaceutical and Biomedical Sciences*, **5**(1), 17-23.
- [20] Latunde-Dada, G. O. (1990). Effect of Processing on Iron Levels and Availability on Nigeria Vegetables. *Journal of Science Food and Agriculture*, **53**(3), 355-361.
- [21] Nair, K. P. P. (2013). The Agronomy and Economy of Turmeric and Ginger. Amsterdam: Elsevier.
- [22] Nanasombat, S. & Teckchuen, N. (2009). Antimicrobial, Antioxidant and Anticancer Activities of Thai Local Vegetables. *Journal of Medicinal Plant Research*, **3**(5), 443-449.
- [23] Ooi, P. S., Rohaida, A. R., Nur Hardy, A. D., Devina, D., Borhan, A. H., Kartini, S., Jupikely, J. S., Abdul Rahman, M. & Alimon, A. R. (2018). Effect of Local Medicinal Herbs as Feed

Additives on Production Performance and Faecal Parameters in Laying Hens. *Malaysian Journal of Animal Science*, **21**(2), 59-67.

- [24] Padhi, L. & Panda, S. K. (2015). Antibacterial Activity of *Eleutherine bulbosa* Against Multidrug-Resistant Bacteria. *Journal of Acute Medicine*, 5(3), 53-61.
- [25] Prasad, R. N., Viswanathan, S., Devi, J. R., Nayak, V., Swetha, V. C., Archana, B. R., Parathasarathy, N. & Rajkumar, J. (2008). Preliminary Phytochemical Screening and Antimicrobial Activity of Samanea saman. Journal of Medicinal Plants Research, 2(10), 268-270.
- [26] Rahardja, D. P., Hakim, M. R. & Lestari, V. S. (2015). Egg Production Performance of Old Laying Hen Fed Dietary Turmeric Powder. World Academy of Science, Engineering and Technology, International Journal of Animal and Veterinary Sciences, 9(7), 748-752.
- [27] Ridzuan, P. M., Hairul Aini, H., Norazian, M. H., Shah, A., Roesnita & Aminah, K. S. (2013). Antibacterial and Antifungal Properties of *Persicaria odorata* Leaf against Pathogenic Bacteria and Fungi. *The Open Conference Proceedings Journal*, 4(1), 71-74.
- [28] Rodriguez-Garcia, A., Hosseini, S., Martinez-Chapa, S. O. & Cordell, G. A. (2017). Multi-target Activities of Selected Alkaloids and Terpenoids. *Mini-Reviews in Organic Chemistry*, 14(4), 272-279.
- [29] Rupasinghe, H. P., Jackson, C. J., Poysa, V., Di Berardo, C., Bewley, J. D. & Jenkinson, J. (2003). Soyasapogenol A and B Distribution in Soybean (*Glycine max* L. Merr.) in Relation to Seed Physiology, Genetic Variability and Growing Location. *Journal of Agricultural and Food Chemistry*, **51**, 5888-5894.
- [30] Saraswati, T. R., Manalu, W., Ekastuti, D. R. & Kusumorini, N. (2013). The Role of Turmeric Powder in Lipid Metabolism and Its Effect on Quality of the First Quail's Egg. *Journal of Indonesia Tropical Animal Agriculture*, 38(2), 123-130.
- [31] Sasongko, P., Laohankunjit, N. & Kerdchoechuen, O. (2011a). Antibacterial Activity of the Essential Oil from *Persicaria odorata* Leaves. *Journal of Agricultural Science*, **42**(2), 105-108.
- [32] Sasongko, P., Laohankunjit, N. & Kerdchoechuen, O. (2011b). Evaluation of Physicochemical Properties of Plant Extracts from *Persicaria odorata*. *Journal of Agricultural Science*, **42**(2), 333-336.
- [33] Sharma, A. D., Kainth, S. & Gill, P. K. (2006). Inulinase Production using Garlic (*Allium sativum*) Powder as a Potential Substrate in *Streptomyces* sp. *Journal of Food Engineering*, **77**(3), 486-491.
- [34] Soetan, K. O. & Oyewole, O. E. (2009). The Need for Adequate Processing to Reduce the Anti-Nutritional Factors in Animal Feeds: A Review. *African Journal of Food Science*, **3**(9), 223-232.
- [35] Starkenmann, C., Luca, L., Niclass, Y., Praz, E. & Roguet, D. (2006). Comparison of Volatile Constituents of *Persicaria odorata* (Lour.) Sojak (*Polygonum odoratum* Lour.) and *Persicaria hydropiper* L. Spach (*Polygonum hydropiper* L.). *Journal of Agricultural and Food Chemistry*, 54(8), 3067-3071.
- [36] Thakuria, P., Nath, R., Sarma, S., Kalita, D. J., Dutta, D. J., Borah, P., Sharma, R., Barman, C. & Hussain, J. (2018). Phytochemical Screening of Medicinal Plants Occurring in Local Area of Assam. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 186-188.
- [37] Usman, H. & Osuji, J. C. (2007). Phytochemical and In Vitro Antimicrobial Assay of the Leaf Extract of Newbouldia Leavis. *African Journal of Traditional, Complementary and Alternative medicines*, **4**(4), 476-480.
- [38] Usman, H., Abdulrahman, F. I. & Usman, A. (2009). Qualitative Phytochemical Screening and In Vitro Antimicrobial Effects of Methanol Stem Bark Extract of *Ficus Thonningii* (Moraceae). African Journal of Traditional, Complementary and Alternative Medicines, 6(3), 275-295.
- [39] Wink, M. (2015). Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines*, 2(3), 251-286.