NJBMB (2022) 37(2) 122-127

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

Phytochemical Profile and Antioxidant Activity of Leaf Essential Oil of *Laggera aurita* (L.) Native to North-central Nigeria

Ismaeel R. Olanrewaju[1*], Usman L. Ajao[1]

[1]*Department of Chemistry, University of Ilorin, Ilorin, Nigeria*

OPEN ACCESS

***CORRESPONDENCE**
Olanrewaju, I.R.
ridwanlanre@gmail.com
+234-806-9315-518

ARTICLE HISTORY
Received: 11/04/2021
Reviewed: 11/05/2021
Revised: 25/05/2021
Accepted: 02/06/2021
Published: 30/06/2022

CITATION
Olanrewaju, I.R and Ajao, U.L. (2022).Phytochemical profile and antioxidant activity of leaf essential oil of *Laggera aurita* (L.) native to North-central Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(2), 122-127

ABSTRACT

Oxidative stress is involved in the pathogenesis of various diseases in humans. The stress is curtailed using synthetic antioxidants whose usage is linked to several side effects. Essential oils have been reported to possess antioxidant activity devoid of any side effect. Their activity is a function of the type of phytochemicals in the oils. The aim of this study is therefore to characterize and evaluate antioxidant activity of essential oil from the leaves of *Laggera aurita* growing in north-central Nigeria. Leaves (500g) of *L. aurita* were pulverized and subjected to hydrodistillation. The antioxidant activity of the oil was evaluated using DPPH radical scavenging assay with ascorbic acid as standard. The hydrodistillation yielded 0.07±0.02% (w/w) of essential oil. GC and GC-MS analysis of the oil show the presence of twenty-three phytochemicals that have β-caryophyllene (27.8%) as the most abundant compound. Other principal constituents of the oil were; β-sesquiphellandrene (12.4%), β-bisabolol (3.4), α-bisabolol (4.4%), α-copaene (9.4%) and calamenene (8.3%). Terpinen-4-ol (2.4%) and α-terpineol (5.7%) were the two monoterpenoids that constituted the oil. The oil scavenged DPPH radical with IC_{50} of 57.13 µl/ml. The activity of the oil was lower than that of ascorbic acid (IC_{50}= 12.84 µl/ml). However, with the activity exhibited by the oil, it has potential to ameliorate oxidative stress, hence, may serve as alternative to synthetic antioxidants after clinical trials.

Keywords: *Laggera aurita, β-caryophyllene, α-copaene, β-sesquiphellandrene*

INTRODUCTION

Oxidative stress plays a leading role in the development of diabetes, cardiovascular diseases, aging, cancer, and atherosclerosis (Gutteridge, 1993; Liguori *et al.*, 2018; Sharifi-Rad *et al.*, 2020). Oxidative stress is usually managed using synthetic antioxidants such as; butylated hydroxyl anisole, butylated hydroxytoluene, propyl gallate, and *tert*-butylhydroxyquinone (Oboh *et al.*, 2011; Muhammed *et al.*, 2013). The rising challenge of the drawbacks associated with the use of synthetic drugs has elicited the need to search for novel antioxidants from plants that could ameliorate oxidative stress with little or no side effect.

Laggera aurita Linn (DC.) (family *Asteraceae*) is an annual shrub growing as a weed in Nigeria and widely grown in sub-Saharan Africa and Southeast Asia (Burkill, 1985; Mevy *et al.*, 2006; Fulata *et al.*, 2017). The plant is used in folklore medicine for the treatment of nasal congestion, asthma, fever, bronchitis, diarrhea, constipation, rheumatism, and strep throat (Egharevba *et al.*, 2010; Julde *et al.*, 2017; Magajia and Malami, 2018). The antinociceptive, hepatoprotective, antioxidant, antiviral, antimalarial and antibacterial properties of crude extracts of the plant have been reported (Egharevba *et al.*, 2010; Dibala *et al.*, 2014; Olurishe and Mati, 2014; Malami *et al.*, 2016). The reported biochemical and biological activities justified its use in folklore medicine. Phytochemical screening of the plant

extracts revealed the presence of terpenoids, flavonoids, polyphenols, steroids and saponins (Dibala *et al.,* 2014; Sheu *et al.,* 2015). The presence of these phytochemicals is responsible for various biochemical and biological properties of the plant.

The plant possesses essential oil with varying chemical composition and antioxidant activity from one geographical location to another. Essential oil from aerial part of *L. aurita* native to Pakistan exhibited antioxidant activity against DPPH radical. The activity was attributed to the presence of α-cadinol, *t*-cadinol, hexadecanoic acid, 9,12-octadecanoic acid and tridecanoic acid in the oil (Shahwar *et al.,* 2012). Similarly, leaf essential oil of the plant grown in Burkina Faso exhibited DPPH radical scavenging and ferric reducing antioxidant activities. The activities were linked to the predominance of *t*-cadinol and α-cadinol in the oil (Mihin *et al.,* 2019). Terpenoids that were earlier reported in the leaf oil of the plant indigenous to Ouagadougou part of Burkina Faso were; Dimethoxy-*p*-cymene, β-Caryophyllene, α-humullene, caryophyllene oxide and longifolene (Mevy *et al.,* 2006). The variations in the composition pattern and antioxidant activity of the oil could be attributed to differences in agroclimatic conditions of the plant locations. It is on this basis that this study aimed to characterize and evaluate antioxidant potential of leaf essential oil of *L. aurita* growing in north-central Nigeria.

MATERIALS AND METHODS

Sample Collection

Leaves of *Laggera aurita* (1800 g) were harvested at the Botanical garden of University of Ilorin, Ilorin, Kwara State, Nigeria. The plant was identified at the Herbarium of Plant Biology Department, University of Ilorin, where voucher specimens were deposited [UILH/002/1087].

Extraction of Essential Oil

Pulverized leaves (500 g) of *L. aurita* were subjected to hydrodistillation for 3 hours in a Clevenger setup based on British Pharmacopoeia specification (British Pharmacopeia specification, 1980). The oil was collected, preserved in a sealed sample tube and stored under refrigeration at 4 ^{o}C until the analyses were carried out.

Gas Chromatography (GC) Analysis of the Oil

GC analyses of the oil was performed on an Orion micromat 412 double focusing gas chromatography system (Agilent 7809B, Brescia, Italy) fitted with two capillary columns coated with CP- Sil 5 and CP-Sil 19 (fused silica, 25 m x 0.25 mm, 0.15 µm film thickness) and flame ionization detector (FID). A volume of 0.2 mL of the oil was injected at a split ratio of 1:30. Hydrogen was used as a carrier gas and the oven temperature was programmed from 50 to 230 ^{o}C at a rate of 3^{o}C/min. Detector and injection temperatures were maintained at 200 ^{o}C and 250 ^{o}C, respectively. FID signal using the GC HP-chemstation software was used to calculate peak area percentage.

Gas Chromatography - Mass Spectrometry (GC/MS) Analysis of the Oil

A Hewlett Packard HP 5890A GC (Agilent 5890A, Brescia, Italy), interfaced with a VG analytical 70-250 s double focusing mass spectrometers was used. The MS operating conditions were: ionization voltage 70 eV, ion source and transfer line temperature was maintained at 230 ^{o}C. The GC operating conditions were identical with those of GC analyses. The MS data were acquired and processed by on-line desktop with a computer equipped with disk memory. The percentage composition of the oil constituents was computed in each case from GC peak areas.

Identification of Constituents in the Oil

The identification of the constituents in the oil was based on (i) comparison of their retention indices (RI), calculated using a homologous series of n-alkanes (C_7–C_{30}, Supelco Bellefonte, PA, USA) under identical experimental conditions, co-injection with standards and compared with those data from Wiley 275 and NIST 08 libraries (ii) comparison of fragmentation pattern in the mass spectra of each constituent with data from Wiley 275 and NIST 08 libraries (Jennings and Shibamoto, 1980; Joulain and Koenig, 1998; Adams, 2007; Usman *et al.,* 2016). The relative quantity of each constituent was calculated based on peak area of the GC (FID response) without using a correction factor.

DPPH Antioxidant Assay of the Oil

The antioxidant potential of the oil was measured in terms of its hydrogen-donating or radical scavenging ability against DPPH, using the method reported by Ilhami, (2009). In the method, 2,2-diphenyl-1-picryl-hydrazil, DPPH, solution (1.5 ml of 10^{-4} M, in 95 % Ethanol) was separately mixed with the oil (1.5) at various concentrations (12.5-200 µl/ml). Each of the mixtures was shaken thoroughly and incubated in the dark for 30 minutes at ambient temperature. The control was prepared using the same procedure without the oil. Absorbance of the solution was measured at 517 nm using UV-spectrophotometer (UV 1600PC, W & J Instrument Co., Ltd, Mudu Town, China). The assay was carried out in triplicate and the results were expressed as mean values ± standard deviation. The concentration of the oil that gave 50 % inhibition (IC_{50}) was calculated from the graph of percentage inhibition against the oil concentration. Ascorbic acid was used as standard. The percentage inhibitions were calculated using the equation:

$$\%\ Inhibition = \frac{A_0 - A_T}{A_0}$$

Where, A_0 is the absorbance of the control sample (containing all reagents except the test compound) and A_T is the absorbance of the test samples.

Statistical Analysis

The extraction of essential oil and antioxidant tests were carried out in triplicates. The mean values were calculated from the three values. The data for various biochemical parameters were expressed as mean ± SD (n = 3) and compared using one-way analysis of variance (ANOVA), followed by Dunnett multiple comparison test with equal sample size test. Values were considered statistically significant at $p < 0.05$. The IC_{50} values were calculated by non-linear regression analysis from the mean values. Statistics was done using SPSS for windows version 10.

RESULTS AND DISCUSSION

Hydrodistillation of pulverized leaves of *L. aurita* yielded 0.07±0.02%(w/w) of essential oil. Table 1 shows the percentage composition, Kovats retention indices and identities of constituents of leaf essential oil of *L. aurita*

In the Table, twenty-one compounds that represented 99.4% of the oil were identified from their mass spectra. Major compounds in the oil were α-terpineol (5.7%), α-copaene (9.4%), β-sesquiphellandrene (12.4%), β-caryophyllene (27.8%), cedrol (4.9%), α-bisabolol (4.4%), β-bisabolol (3.8%) and calamenene (8.3%). Terpinen-4-ol (2.4%), α-cedrene (1.8%), α-calacorene (1.5%), α-bergamotene (1.2%), β-cadinene (2.8%), β-himachallene (1.6%), viridiflorol (2.6%), α-bergamotene (1.4%), α-bergamotol (1.4%) and geranyl-α-terpinene (2.8%) were present in appreciable quantities in the oil. Terpenoids that were identified in significant amounts in the oil were farnesene epoxide (0.5%) and humullene-1,2-epoxide (0.5%). Stearic (1.7%) and oleic (1.9%) acids were the non-terpenic compounds found in the oil. The predominance of β-caryophyllene implies that the oil was of β-caryophyllene chemotype. Dimethoxy-*p*-cymene and α-cadinol chemotypes were earlier reported for the essential oil from leaves and aerial parts of the plant native to Burkina Faso and Pakistan respectively (Mevy *et al.,* 2006; Shahwal *et al.,* 2012). The variation in the chemotype of the oil could be linked to differences in agroclimatic conditions of the plant locations.

Table 1. Chemical composition (%) of leaf essential oil of *L. aurita*

Compound	% Composition	RI [a]	RI[b]	Mass spectra data
Terpinen-4-ol	2.4	1175	1177	43,69,**71**,93,111
α-Terpineol	5.7	1143	1144	43,**59**,81,93,107
α-Copaene	9.4	1376	1376	105,119,**161**,189,204
α-Cedrene	1.8	1462	1462	41,69,**94**,107,148
α-Calacorene	1.5	1551	1551	119,129,142,**157**,200
α-Bergamotene	1.2	1436	1435	55, 79,**93**, 119, 161
β-Sesquiphellandrene	12.4	1532	1539	**69**,77,93,109,204
β-Cadinene	2.8	1519	1518	69,91,119,**161**,204
β-Caryophyllene	**27.8**	1418	1418	69,**93**,105,133,204
β-Himachalene	1.6	1499	1499	93,105,**119**,134,204
Viridiflorol	2.6	1590	1591	81,93,**109,**121,133
Farnesene epoxide	0.5	1458	1455	93,**119,**161,134,147
Cedrol	4.9	1596	1598	43,**95**,121,150,207
Humulene-1,2-epoxid	0.5	1599	1592	**43**,61,81,96,109
β-Bisabolol	3.8	1668	1662	69,**82**,93,111,119
α-Bisabolol	4.4	1683	1685	69,**109**,119,134,147
α-Bergamotol	1.4	1673		68,79,**93,**107,119
Calamenene	8.3	1521	1498	133,147,**161**,179,189
Geranyl-α-terpinene	2.8	-	1695	**69**,93,132,187,272
Stearic acid	1.7	2141	2139	**43**,60,73,85,98
Oleic acid	1.9	2175	2144	55,69,**83**,97,123
Total	**99.4%**			

Compounds are listed in order of elution from fused silica capillary column coated on CP-Sil 5; RI[a] = Literature Retention Indices, RI[b] = Calculated Retention Indices, Bolded name = Chemotype.

It has been established that synthases of the most abundant mono- and sesquiterpenoids in an essential oil usually aid the biosynthesis of all terpenoids in the oil (Trapp and Croteau, 2001; Degenhardt *et al.,* 2009; Usman *et al.,* 2016). The predominance of α-terpineol and β-caryophyllene therefore implies that their synthases facilitated the transformation of geranyl, neryl and farnesyl pyrosphosphates to all mono- and sesquiterpenoids in the leaf of the plant via cationic intermediates. The activities of

the synthases, which are dictated by agroclimatic conditions, determine the types and quantities of terpenoids in an essential oil. Terpinen-4-ol and α-terpineol were the only monoterpenoids that constituted the oil. Their biosyntheses were mediated by α-terpineol synthase. The fewer number of monoterpenoids in the oil implies that the agroclimatic condition did not favour the activity of α-terpineol to facilitate the biosynthesis of more monoterpenoids. Meanwhile, dimethoxy-*p*-cymene, linalool, *p*-menth-2-en-1-ol, terpinen-4-ol, *p*-cymen-8-ol, α-terpineol, trans-carveole, nerol, thymol, thymol methyl ether and isothymol methyl ether were monoterpenoids that constituted the leaf essential oil of the plant indigenous to Burkina Faso (Mevy *et al.*, 2006). Their biosynthesis in the leaf was aided by dimethoxy-*p*-cymene synthase whose activity was more favoured by the agroclimatic conditions of Burkina Faso and aided the formation of more monoterpenoids in the oil.

The major sesquiterpenoids in the oil were α-copaene, β-sesquiphellandrene, β-caryophyllene, cedrol, α-bisabolol, β-bisabolol and calamenene. β-Caryophyllene synthase aided their biosynthesis in the leaf of the plant. The synthase also mediated the biosynthesis of β-caryophyllene, longipinene, α-himachallene, α-humullene and caryophyllene oxide that were the principal constituents in the leaf oil of the plant indigenous to Burkina Faso. Calamenene, α-copaene, β-sesquiphellandrene, cedrol, α-bisabolol and β-bisabolol that constituted the oil of the Nigerian grown *L. aurita* were not identified in the leaf oil of the plant native to Burkina Faso. In contrast, the oil of the plant growing in Nigeria did not contain longipinene, α-humullene, α-himachallene and caryophyllene oxide that were the major compounds in the oil of the plant native to Burkina Faso. The absence of some mono- and sesquiterpenoids in the oils might be attributed to the effect of agroclimatic conditions of the plant locations on the activity of β-caryophyllene synthase.

Quantitatively, terpinen-4-ol, α-terpineol, β-caryophyllene and β-humullene were constituents of leaf essential oils of *L. aurita* indigenous to Nigeria and Burkina Faso. However, the compounds were of greater quantities in the oil than the oil of the plant grown in Burkina Faso. The lower quantities of the compounds in the oil could be linked to early termination of their reactive intermediates during their biosyntheses by agroclimatic conditions of Burkina Faso (Iijima *et al.*, 2004).

The oil exhibited antioxidant activity against DPPH radical and its activity was concentration dependent (Figure 1).

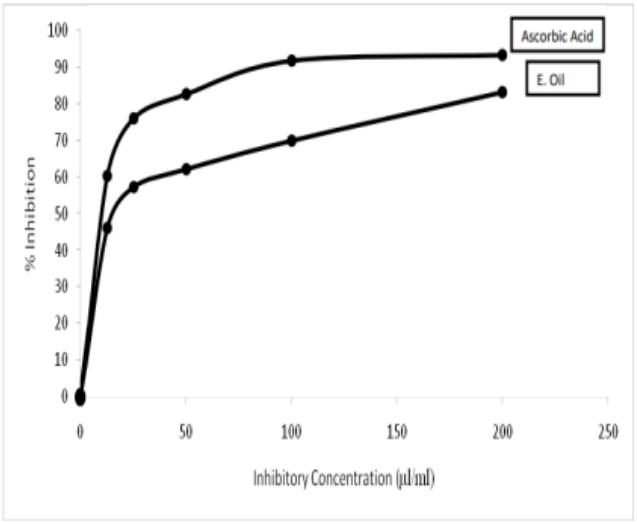

Figure 1. DPPH radical scavenging potential of leaf essential oil of *L. aurita* and ascorbic acid

The DPPH radical scavenging activity of the oil ranged from 44.6 to 80.4%. The activity of the oil increased steadily as concentration increases with IC_{50} of 57.13 µl/ml. Meanwhile, the oil exhibited lower activity than ascorbic acid (IC_{50} = 12.84 µl/ml) that was used as standard. The antioxidant activity of bisabolol, α-terpineol, β-caryophyllene have been established (Braga *et al.*, 2009; Bicas *et al.*, 2011; Calleja *et al.*, 2013). Thus, the antioxidant activity of the oil could therefore be linked to the presence of α-terpineol, β-caryophyllene, α-bisabolol and β-bisabolol.

CONCLUSION

The chemical composition of leaf essential oil of *L. aurita* native to north-central Nigeria is reported for the first time. The composition of the oil differs from the leaf oil of the plant grown in Burkina Faso but both oils were of β-caryophyllene chemotype. The oil exhibited antioxidant activity that could be linked to the synergistic action of the constituents. Hence, the oil could ameliorate oxidative stress after clinical trials.

AUTHORS' CONTRIBUTIONS

Conceptualization, Methodology and Writing of Original Draft: IRO; Methodology, Writing-review and Editing: ULA. Both Authors have read and approved the manuscript.

FUNDING STATEMENT

There was no grant from any funding agency for the research.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest in this manuscript.

ACKNOWLEDGEMENT

The authors acknowledge Mr. Bolu at the Herbarium of the department of Plant Biology, University of Ilorin, who assisted in the plant identification.

REFERENCES

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (Vol. 456, pp. 544-545). Carol Stream: Allured publishing corporation.

Bicas, J. L., Neri-Numa, I. A., Ruiz, A. L., De Carvalho, J. E., Pastore and G. M. (2011). Evaluation of the antioxidant and antiproliferative potential of bioflavours. *Food and Chemical Toxicology*, 49(7), 1610-1615.

Braga, P. C., Sasso, M. D., Fonti, E. and Culici, M. (2009). Antioxidant activity of bisabolol: inhibitory effects on chemiluminescence of human neutrophil bursts and cell free systems. *Pharmacology*, 83(2), 110 – 115.

British pharmacopoeia, II, .(1980). pp. 109, HM, stationary office, London.

Burkill, H. M. (1985). The useful of plants of West/Tropical Africa. *Royal Botanical Gardens, Kew*, 1(481), 452 – 453.

Degenhardt, J., Ko¨llner, T. G. and Gershenzon, J. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, 70(15-16), 1621-1637.

Calleja, M. A., Vietes, J. M., Montero-Meterdez, T., Torres, M. I., Faus, M. A., Gil, A. and Suarez, A. (2013). The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *British Journal of Nutrition*, 109(3), 394-401.

Egharevba, O. H., Oladosu, P., Okhale, E. S., Ibrahim, I., Folashade, K. O., Okwute, K. O., Okogun, I. J. (2010). Preliminary antituberculosis screening of two Nigerian *Laggera* species (*Laggera pterodonta* and *Laggera aurita*). *Journal of Medicinal Plant Research*, 4(12), 1235 - 1237.

Fulata A., Usman H., William A., Ibrahim S. and Zadva A. I. (2017). Phytochemical, analgesic and anti-inflammatory evaluation of the crude ethanol and n-hexane extracts of the aerial parts of *Laggera aurita* Linn. *Chemistry Research Journal*, 2(5), 22 – 28.

Gutteridge, J. M. (1993). Free radicals in disease processes: a compilation of cause and consequence. *Free Radical Research Communications*, 19(3), 141-158.

Iijima, Y., Davidovich-Rikanati, R.,Fridman, E., Gang, D. R. (2004). The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phynyl propenes in the peltate glands of three cultivars in Brasil. *Plant Physiology*, 136(3), 3724 - 3736.

Ilhami, G. (2009). Antioxidant activity of L-adrenaline: A structure activity insight. *Chemico-Biological Interactions*, 179(2&3): 71-80.

Jennings W. and Shibamoto T. (1980). *Qualitative analysis of flavor volatiles by gas capillary chromatography*. Academic press, New York. pp. 68 – 109.

Joulain D. and Koenig W. A. (1998). *The atlas of spectra data of sesquiterpene hydrocarbon*. E. B. Verlay Hamburg, Germany. pp. 112 – 153

Julde, S., Kyari, H. and Malami, S. (2017). Assessment of serum parameters after 28 days oral daily administration of methanol leaf extract of *Laggera aurita* (Linn.) in Wistar rats. *Tropical Journal of Natural Products Research*, 1, 251 – 254.

Dibala, C. I., Konaté, K., Diao, M., Ouédraogo, M. and Dicko, M. H. (2014). Phytoconstituents analysis, antioxidant capacity and antimicrobial properties of extracts from *Laggera aurita* L. (*Asteraceae*). *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 0975-1491.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D. and Abete, P. (2018). Oxidative stress, aging and diseases. *Clinical Interventions in Aging*, 13, 757-772. https://doi.org/10.2147/CIA.S158513

Magajia, S. Y. and Malami, S. (2018). Antiplasmodial properties of methanol leaf extracts of *Laggera aurita* Linn. in experimental animals. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 7(2), 46 - 53.

Malami, S., Kyari, H., Danjuma, N. M., Ya'u, J. and Hussaini, I. M. (2016). Anticonvulsant properties of methanol leaf extract of *Laggera aurita* Linn. F. (Asteraceae) in laboratory animals. *Journal of Ethnopharmacology*, 191, 301 - 306.

Mevy, J. P., Bousquet-Melou, A., Greff, S., Millogo, J. and Fernandez, C. (2006). Chemical composition of the volatile oil of *Laggera aurita* Schulz. from Burkina-Faso. *Biochemical Systematics and Ecology*, 34(11), 815-818.

Mihin, H. B., Somda, M. K., Kabore, D., Sanon, S., Akakpo, A. Y., Traore, A. S. and Ouattara, A. S. (2019). Biopreservation of meat by using antimicrobial properties of essential oil from *Laggera aurita* in Burkina Faso. *Advances in Nutrition and Food Science*, 2019(02), 1-13.

Muhammed, S. A., Yaqub, A. G., Sanda, K. A., Nicholas, A. O., Arastus, W., Muhammed, M. and Abdullahi, S. (2013). Review on diabetes, synthetic drugs and glycemic effects of medicinal plants. *Journal of Medicinal Plant Research*, 7(36), 2628-2637.

Oboh, G., Ademiluyi, A. O. and Faloye, Y. M. (2011). Effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against α-amylase and α-glucosidase activities in vitro. *Journal of Medicinal Food*, 14(10), 1152–1158. https://doi.org/10.1089/jmf.2010.0194

Olurishe, T. O. and Mati, F. G. (2014). Anti-hyperanalgesic potentials of *Laggera aurita* in Swiss albino mice,

Pakistan Journal of Pharmaceutical Science, 27(1), 169 - 172.

Shahwar, D., Ahmad, N., Khan, M. A., Ullah, S. and Ahmad N. (2012). Chemical composition and biological activities of the essential oil of *Laggera aurita* Linn. (DC.) grown in Pakistan. *Turkish Journal of Biochemistry*, 37(3), 329 - 335.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., Calina D., Cho W. C. and Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694-699. https://doi.org/10.3389/fphys.2020.00694

Sheu, A., Olurishe, T. O., Zezi, A. and Ahmed, A. (2015). Analgesic and anti-inflammatory activities of saponin and flavonoid fractions of *Laggera aurita* in mice and rats. *Nigerian Journal of Pharmaceutical Sciences*, 15(1), 60 – 69.

Trapp, S. C. and Croteau, R.B. (2001). Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genetics*, 158(2), 811–832.

Usman, L. A., Agboola, T. A., AbdulWaheed, J. O., Ismaeel, R. O., Ogundele, V. A. and Ibrahim, A. (2016). Phytochemical profile of fruit and leaf essential oils of *Thuja orientalis* grown in North central Nigeria. *Nigerian Journal of Pure and Applied Sciences*, 29(2), 2968 - 2976.