Azhar Int J Pharm Med Sci 2023; Vol 3 (2):70-76
(Research Article)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Phytochemical Profile and Antioxidant Capacity of *Ficus natalensis* Subsp. *leprieurii* (miq) Cultivated in Egypt: *In-vitro* Study

Shaimaa E.A. Elish [1, *], Mostafa H. Baky [1], and Abeer Temraz [2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Badr city, Cairo, Egypt.
[2] Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
***** Correspondence: shaimaa-essam@eru.edu.eg

Article history: Received 2022-08-21 Revised 2022-11-10 Accepted 2022-12-01

Abstract: *Ficus natalensis* Subsp. *leprieurii* (miq) (*F. natalensis*) is an evergreen tree belonging to the family Moraceae and is widely distributed in Africa. The leaves of *F. natalensis* are well-known for their nutritional value and health benefits and were used traditionally to treat several ailments. The objective of this study was to identify the major classes of phytoconstituents, isolate some of them, and investigate the free radical scavenging potential of *F. natalensis* leaves. The leaves methanol extract of *F. natalensis* revealed an abundance of flavonoids, tannins, carbohydrates and/or glycosides, sterols, saponins, and triterpenes. Additionally, the total phenolic content (TPC) of *F. natalensis* methanol extract was 54.8 mg GAE/g and the total flavonoid content (TFC) was 14.78 mg RE/g. Moreover, three triterpenoid saponins were isolated from the methanol extract using different chromatographic techniques and identified by using 1D NMR together with mass spectrometry as 3-methoxy-quinovic acid-28-*O*-*α*-L-rhamnopyranoside ester (**1**), quinovic acid 3-*O*-methoxy (**2**), and 3-*O*-*α*-L-rhamnopyranoside-quinovic acid (**3**). Finally, the leaves methanol extract of *F. natalensis* revealed 2,2-diphenyl-1-picryhydrazyl (DPPH•) free radical scavenging capacity with IC_{50}=80.22 µg/mL, compared to that of standard rutin (37.5 µg/mL) which suggests that *F. natalensis* could be a good source for phytochemical leads with higher antioxidant potential and health benefits.

Keywords: *Ficus natalensis*; Moraceae ; Antioxidant capacity; DPPH; Saponin; Phytochemical screening.

1. INTRODUCTION

Plants are considered an important source of phytochemicals that play a pivotal role in the discovery of bioactive compounds beneficial for human health. Numerous secondary metabolites produced by plants, such as flavonoids, alkaloids, saponins, steroids, terpenoids and have numerous therapeutic and health benefits [1]. Family Moraceae, also known as the fig family, is a family of flowering plants widely distributed in tropical and subtropical regions and comprising about 38 genera and over 1100 species [2]. Family Moraceae involves the genus *Ficus*, which includes about 850 species generally known as fig trees or figs [3]. Several *Ficus* species are used in traditional medicine to treat constipation, dyspepsia, rheumatic disease, dysentery, parasitic worms, hypertension, and diabetes [3]. The evergreen *F. natalensis* tree is found throughout Africa and is used in traditional medicine alone or mixed with other medicinal plants to treat several ailments. Its aerial parts and roots exhibited activities for the treatment of cold, cough, sore throat, and dysentery [4]. Several phytochemicals were reported from it such as Stigma-4-en-3-one[5, 6], *β*- Sitosterol[6, 7], and Phytol [8, 9]. Moreover, biological studies on *F. natalensis* leaves revealed its anti-microbial effect [10] and alleviating cadmium chloride testicular disruptions[11]. Hence, the main goal of this study was to assess the antioxidant activity of *F. natalensis* leaves cultivated in Egypt and to investigate their phytochemical profile.

Cite this article: Elish SE., Baky MH., Temraz A. Phytochemical Profile and Antioxidant Capacity of *Ficus natalensis* Subsp. leprieurii (miq) Cultivated in Egypt: *In-vitro* Study. Azhar International Journal of Pharmaceutical and Medical Sciences, 2023; 3(2):70-76. Doi: 10.21608/AIJPMS.2023.157607.1163

DOI : 10.21608/AIJPMS.2023.157607.1163

2. METHODS

2.1. Plant material

In August 2019, the leaves of *Ficus natalensis* Subsp. *leprieurii* (miq) were taxonomically authenticated by Taxonomy Department at Faculty of Science, Cairo University, Egypt. Then, the plant material was collected from the Horticultural Research Institute in Giza, Egypt, and dried in the shade. A voucher specimen (Fn-2019) was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt. The dried material was ground and kept in a closed glass container until extraction.

2.2. Phytochemical screening

The crude powder of *F. natalensis* leaves was screened for the presence of various classes of phytoconstituents using standard procedures described by Harborne, and Trease, and Evans [12-14].

2.3. Estimation of TP and TF content

The total phenolic content of *F. natalensis* leaves methanol extract was determined using Micro plate Follin-Ciocaltau's analysis [15], while its flavonoid content was determined colorimetrically using $AlCl_3$ assay [16].

2.4. Antioxidant capacity estimation

The DPPH• free radical scavenging ability was evaluated as described by Boly et al. [17] and Brand-Williams et al. [18]**.** The data were expressed as means ± SD using the following equation:

$$Percentage\ inhibition = \frac{\text{Average absorbance of blank-average absorbance of the test}}{\text{Average absorbance of blank}} \times 100$$

2.5. General experiment

The NMR analysis was carried out at Faculty of Pharmacy, Cairo University, Egypt, using a Bruker High Performance Digital FT-NMR Spectrometer Advance III 400 MHz for ^1H & 100 MHz for C [13]. The samples were dissolved in DMSO-d_6, and tetramethyl silane (TMS) was utilized as an internal standard. Chemical shifts and coupling constants (*J*) were represented in ppm and Hertz, respectively. The mass analysis was carried out at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt, using Thermo Scientific GCMS model ISQ. Electron ionization (EI) fragmentation mode at 70 eV was utilized in the Mass spectroscopy analysis to evaluate the purity of the compounds and to investigate the individual fragments. Chloroform ($CHCl_3$) and methanol (MeOH) were used as solvents for column chromatography. Glass columns with different dimensions were utilized for chromatography, and Silica gel F254 (E. Merck, Darmstadt, Germany) was used as a stationary phase.

2.6. Extraction and fractionation

The dried ground plant material (2.5 kg) was extracted with (3X4L) of methanol at room temperature until exhaustion. The total extracts were collected and evaporated at 45 °C under vacuum using rotary evaporator to yield 250 g dry methanol extract. The dry extract was suspended in 350 mL of water and partitioned using three organic solvents in order of polarity, namely petroleum ether, chloroform, and *n*-butanol, to yield four fractions: petroleum ether (44 g), chloroform (38.5 g), *n*-butanol (50 g) and water residue (50 g). To precipitate crude saponins, the *n*-butanol fraction was dissolved in 100 mL methanol and poured with continuous stirring into 500 mL acetone, yielding an acetone-soluble portion (contains phenolics) and an acetone precipitate (contains saponins). The precipitated phytoconstituents (25 g) were recovered by filtration and subjected to a silica gel column using a gradient elution system consisting of $CHCl_3$ and MeOH. Eluted fractions were collected based on the similarity shown on TLC plates using $CHCl_3$: MeOH: H_2O (7:3:0.5 v/v) solvent system and ethanol-sulphuric acid spray reagent to give 19 collective fractions. Compound 1 was obtained from collective fraction (12) after being subjected to further silica columns, while compounds 2 and 3 were obtained from collective fraction (6) after purification using silica gel columns followed by sephadex column using MeOH as an eluent.

3. RESULTS

3.1. Phytochemical screening

The preliminary phytochemical investigation results showed the presence of carbohydrates, phenols, tannins, flavonoids, saponins, phytosterols, and terpenoids.

3.2. TPC

The phenolic content of *F. natalensis* leaves methanol extract was evaluated as gallic acid equivalent GAE calculated from gallic acid

calibration curve. The measured phenolic content was 54.8 mg of GAE/g (Figure 1).

3.3. TFC

The flavonoid content of *F. natalensis* leaves methanol extract was 14.78 mg of RE/g using rutin calibration curve (Figure 2).

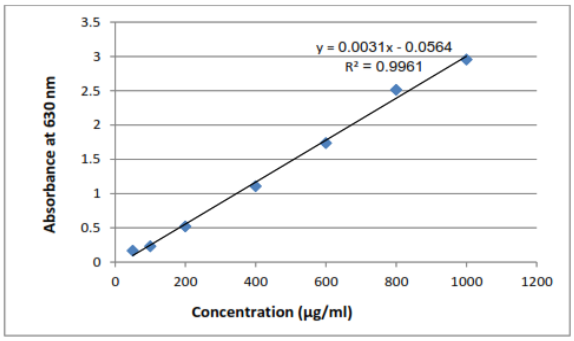

Figure 1. Calibration curve of gallic acid.

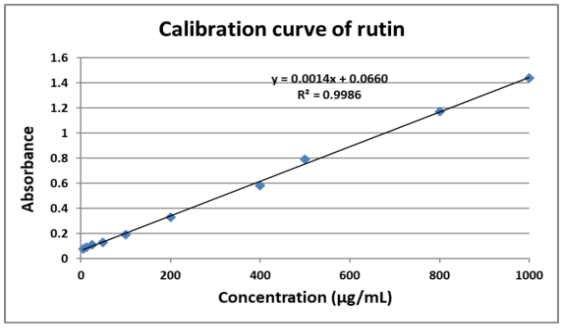

Figure 2. Calibration curve of rutin.

3.4. Antioxidant assay

The antioxidant activity of the methanol extract of *F. natalensis* leaves was investigated in a concentration-dependent manner against DPPH• free radicals; the results revealed 50% inhibition (IC_{50}) at a concentration of 80.22 µg/mL compared to 37.5 µg/mL for the standard rutin (Figure 3).

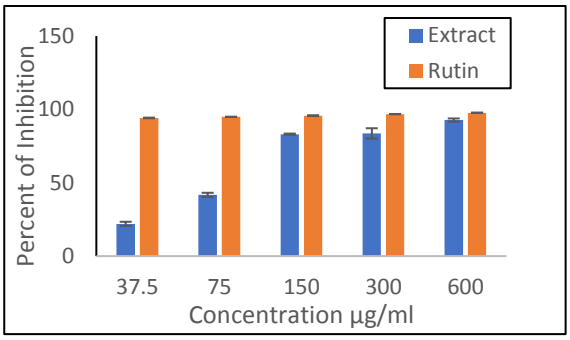

Figure 3. DPPH free radicals scavenging activity of Ficus natalensis methanol extract versus standard rutin.

3.5. Isolated compounds

Chromatographic separation of *n*-butanol fraction of *F. natalensis* yielded three triterpenoid saponins that were identified as 3-methoxy-quinovic acid-28-*O*-*α*-L-rhamnopyranoside ester **(1)**, quinovic acid 3-*O*-methoxy **(2)**, and 3-*O*-*α*-L-rhamnopyranoside-quinovic acid **(3)**, (Figure 4).

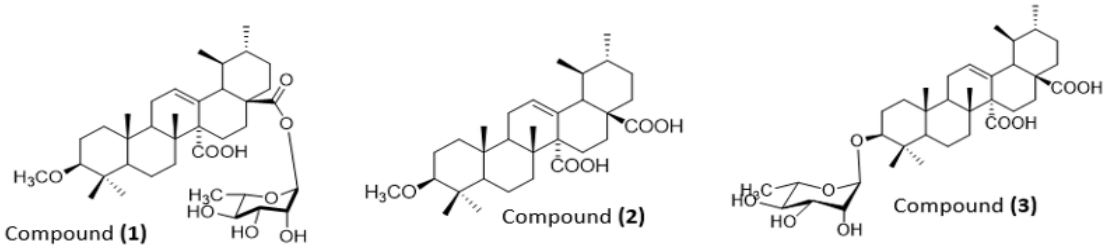

Figure 4. Chemical structures of isolated saponins from *Ficus natalensis* methanol extract.

Compounds (**1-3**) were isolated as amorphous yellowish-white powders (7, 5, and 8mg, respectively) that produced pink spots when sprayed with ethanol-sulphuric acid reagent. On a TLC plate, the retention factors (R_f) of compounds **(1-3)** were 0.35, 0.30, and 0.43 using a solvent system of $CHCl_3$: MeOH: H_2O (7:3:0.5 v/v). Based on EI and ^{1}H- and ^{13}C-NMR spectral data, the structures of the three compounds were elucidated. EI mass spectrum of compound **(1)** showed a molecular ion peak $[M]^+$ at 646.91 *m/z*, compatible with $C_{37}H_{58}O_9$ molecular formula, while the spectra of compounds **(2)** and **(3)** showed $[M]^+$ at 500.88and 631.35*m/z*, respectively which were compatible with $C_{31}H_{48}O_5$and $C_{36}H_{55}O_9$ molecular formulas. Spectral data of ^{1}H- and ^{13}C-NMR for the three compounds were recorded in Table 1. Based on these findings that were consistent with published data, Compounds (1-3) were identified as 3- methoxy -quinovicacid-28-*O*-*α*-L-rhamnopyranoside ester, quinovic acid 3-*O*-methoxy, and 3-*O*-*α*-L -rhamnopyranoside-quinovic acid, respectively[19-21].

Table 1. ^{1}H -and ^{13}C-NMR spectroscopic data of compounds (1-3) in DMSO-*d6*

Carbon No.	**Compound 1**		**Compound 2**		**Compound 3**	
	δ ^{1}H (*J*=Hz)	**δ ^{13}C**	**δ ^{1}H (*J*=Hz)**	**δ ^{13}C**	**δ ^{1}H (*J*=Hz)**	**δ ^{13}C**
1	1.72(m)	38.54	1.53(m)	38.53	1.52(m)	40.0
2	2.01(m)	28.82	1.84(m)	28.84	1.96(m)	28.36
3	3.17(d, *J*=12)	74.60	3.00(d, *J*=12)	70.21	3.50(d, *J*=12)	81.37
4		40.70		42.98		40.92
5		55.48		56.7		57.39
6		19.05		20.65	1.46(m)	19.4
7		37.84	2.08(m)	35.93	1.50(m)	37.9
8		40.76		40.67		42.65
9		48.1		47.59		49.04
10		38.0		36. 34		36.96
11		22.55		22.57		22.54
12	5.32(t)	129.12	5.32(t)	127.71	5.32(t)	130.11
13		132.06		130.10		136.38
14		57.4		63.50		43.23
15	1.61(m)	29.02	1.55(m)	29. 01	1.59(m)	29.27
16	1.86(s)	29.15	2.00(s)	29.27	1.96(s)	29.46
17		42.47		48.25		49.8
18		39.09		50.05		51.82
19	1.36(m)	38.73		39.8	1.36(m)	39.28
20		41.0		38.3		40.53
21	1.22(s)	30.25		30.26	1.21(s)	30.24
22	1.24(s)	36.51	1.24(s)	37.0	1.24(s)	37.0
23	0.84(s)	25.80	0.70(s)	26.99	0.84(s)	28.6
24	0.90(s)	17.64	0.90(s)	17.23	0.89(s)	17.90
25	0.88(s)	16.22	0.86(s)	14.41	0.88(s)	14.40
26	0.87(s)	22.85	0.84(s)	22.18	0.87(s)	22.85
27		182.60		178.43		182.22
28		178.50		176.99		176.29
29	0.99(s)	22.93	0.99(s)	22.57	0.97(s)	25.57
30	1.01(s)	23.71	1.01(s)	23.70	1.07(s)	27.00
3-*O*- Methoxy	3.17(s)	55.48	3.51(s)	51.01		
O- Rhamnose						
1'	5.19(d *J* = 1.5)	100.59			5.41(d *J* = 1.5)	99.98
2'	3.96(dd *J* = 3.4, 1.7)	74.55			3.56(dd *J* = 3.4, 1.7)	71.46
3'	3.62(dd *J* = 9.7, 3.2)	74.60			3.89(dd *J* = 9.7, 3.2)	72.22
4'	3.97(t *J* = 9.4)	69.75			3.45(t *J* = 9.4)	71.63
5'	5.34(dq *J* = 9.5, 6.0)	67.88			3.89(dq *J* = 9.5, 6.0)	72.32
6'	4.13(d *J* = 6.0)	17.54			1.06(d *J* = 6.0)	17.90

4. DISCUSSION

The phytochemical investigation of a methanol extract of *F. natalensis* leaves revealed the presence of different phytochemical classes, mainly phenolics, and saponins. In accordance with the phytochemical screening, the TPC and TFC of *F. natalensis* leaves methanol extract revealed the presence of abundant amounts of phenolics and flavonoids. Additionally, three pure triterpenoid saponins were isolated from the extract. ^{1}H-NMR spectrum of compound **(1)** in DMSO-*d6* revealed the presence of six signals at δ_H 0.84, 0.90, 0.88, 0.87, 0.99, and 1.01, corresponding to six angular methyls at carbons-23, 24, 25, 26, 29, and 30, respectively. At δ_H 5.32 (t) and 3.17 (dd, *J* = 12.0, 4 Hz), two additional signals for H-12 and 3 were detected, which are characteristic of the triterpenoid skeleton. Moreover, three singlet signals corresponding to H-22, 21, and 16 were observed at δ_H1.24, 1.22, and 1.86, respectively. The aglycone part signals, H-19, 15, 2, and 1 were observed at δ_H 1.36, 1.61, 2.01, and 1.72. ^{13}C-NMR spectrum showed a signal for ethylenic carbon-12 at δ_C 129.12, and a signal for quaternary carbon-13 at δ_C 132.06. Additionally, the two signals specific to carboxylic carbons -27 and 28 appeared at δ_C 182.60 and 178.50. Both ^{1}H- and ^{13}C-NMR data revealed the existence of one anomeric signal at δ_H 5.19 (d *J* = 1.5) and at δ_C 100.59 ppm, corresponding to H-1' and C-1' that confirm the existence of one rhamnose moiety attached to the aglycone. Furthermore, ^{1}HNMR revealed the presence of a singlet signal at δ_H 3.17, which is characteristic of three protons in the methoxy group, and this was verified by the appearance of a carbon signal at C 55.48 in the ^{13}C-NMR spectrum. The attachment of methoxy group was confirmed to be attached at C-3 from the upfield shift of the oxygenated carbon 3 to δ_C 74.60. ^{1}H- and ^{13}C-NMR data (in DMSO-*d6*) revealed that the skeleton of the aglycone was a ursane-type triterpene, with 2 carboxylic groups at carbon-27 and 28 and an olefinic bond at carbon-12, which were compatible with quinovic acid structure with an additional methoxy group and a rhamnose moiety attached to C-28. Comparing these data with previously published literature, compound **(1)** was identified as 3-methoxy-quinovic acid-28-*O*-*α*-L-rhamnopyranoside ester[19-21].

Compound **(2)** exhibited identical ^{1}H- and ^{13}C-NMR data to compound (**1**), excluding the sugar signals, as shown in Table 1. Compound (**2**) was identified as quinovic acid 3-*O*- methoxy based on its NMR spectral data, which matched published literature, and its mass spectral data. Also, compound (**3**) showed the same NMR spectral data as compound (**1**) except the absence of methoxy protons and carbon signals in addition to the attachment of rhamnose moiety at carbon-3, which was proved by spectral data of H-3 at 3.50 ppm and C-3 carbon at 81.37 ppm, which coincide with the previously reported literature[19-21]. From these data and its mass spectrum, compound **(3)** was identified as 3-*O*-*α*-L-rhamnopyranoside-quinovic acid.

Investigation of the antioxidant capacity of *F. natalensis* leaves methanol extract revealed free radical scavenging capacity against DPPH• radicals with IC_{50}=80.22 µg/mL, compared to that of the standard antioxidant rutin (37.5 µg/mL). This antioxidant activity could be attributed to the abundance of free radical-scavenging phytochemicals, such as phenolic compounds and flavonoids.

5. CONCLUSIONS

The phytochemical investigation of *F. natalensis* leaves methanol extract allowed the isolation and identification of triterpenoid saponins and showed high values of TPC and TFC. The extract revealed good free radical scavenging activity against DPPH• free radicals which could be referred to phenolic and flavonoid content of the plant. These results indicate that *F. natalensis* could be a good source of phytochemical leads that can be used to prevent or reduce the development of various oxidative stress-related disorders. Finally, owing to these promising results, more deep phytochemical investigations with *in-vivo* biological studies are recommended.

Funding: NA.

Acknowledgments: Sincere thanks is extended to Faculty of Pharmacy, Egyptian Russian University for providing us with the laboratory facilities necessary to complete this work. We would like to thank Prof. Dr. Rim Hamdy, Taxonomy department, Faculty of Science, Cairo University, for her efforts in identifying the plant material.

Conflicts of Interest: All authors declared no conflict of interest.

Author Contribution: Shaimaa E.A. Elish: Methodology, Writing - review editing. Mostafa H. Baky: Supervision, Conceptualization, Methodology, Investigation, Writing - review

editing. Abeer Temraz: Supervision, Conceptualization, Investigation, Writing - review editing.

REFERENCES

1. Baky MH, Kamal AM, Haggag EG, Elgindi MR. Flavonoids from *Manilkara hexandra* and antimicrobial and antioxidant activities. Biochemical Systematics and Ecology. 2022;100:104375.

2. Somashekhar M, Nayeem N, Sonnad B. A review on family Moraceae (Mulberry) with a focus on *Artocarpus* species. World J Pharm Pharm Sci. 2013;2:2614-21.

3. Olaokun OO, McGaw LJ, Eloff JN, Naidoo V. Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African *Ficus* species (Moraceae) used traditionally to treat diabetes. BMC Complementary and Alternative medicine. 2013;13(1):1-10.

4. Huh MK, Huh HW. Contemporary Plant Systematics. Contemporary Plant Systematics. 222, 1991. Journal of plant research. 1999;112(1108):437-42.

5. Liu B, Jian L, Chen G, Song X, Han C, Wang J. Chemical constituents and in vitro anticancer cytotoxic activities of *Polyalthia plagioneura*. Chemistry of Natural Compounds. 2014;49(6):1172-4.

6. Awolola GV. Phytochemical analyses and biological activities of four South African *ficus* species (moraceae) 2015.

7. Rasoanaivo LH, Wadouachi A, Andriamampianina T, Andriamalala S, Razafindrakoto E, Raharisololalao A, et al. Triterpenes and steroids from the stem bark of *Gambeya boiviniana* Pierre. J Pharmacogn Phytochem. 2014;3(1):68-72.

8. Sonibare MA, Sonibare OO, Akharame OE, Soladoye MO. Chemical Composition of Essential Oils of *Ficus elasticoidies* De Wild., *Ficus ovata* Vahl and *Ficus natalensis* subsp. *leprieurii* (Miq.) CC Berg from Nigeria. Journal of Essential Oil Bearing Plants. 2009;12(3):282-6.

9. Ogunwande IA, Jimoh R, Ajetunmobi AA, Avoseh NO, Flamini G. Essential oil composition of *Ficus benjamina* (Moraceae) and *Irvingia barteri* (Irvingiaceae). Natural Product Communications. 2012;7(12):1934578X1200701233.

10. Tkachenko H, Buyun L, Terech-Majewska E, Osadowski Z. In vitro antimicrobial activity of ethanolic extracts obtained from *Ficus* spp. leaves against the fish pathogen Aeromonas hydrophila. Archives of Polish Fisheries. 2016;24(4):219-30.

11. Elish SE, Sanad FA, Baky MH, Yasin NA, Temraz A, El-Tantawy WH. *Ficus natalensis* extract alleviates Cadmium chloride-induced testicular disruptions in albino rats. Journal of Trace Elements in Medicine and Biology. 2022;70:126924.

12. Harborne J. Phytochemical methods London Chapman and Hall. Ltd; 1973.

13. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.

14. Trease G, Evans W. Pharmacognosy, 11th end, brailliere tindall. London; 1989.

15. Attard E. A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. Open Life Sciences. 2013;8(1):48-53.

16. Kiranmai M, Kumar CM, Mohammed I. Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011;2(3):254-61.

17. Boly R, Lamkami T, Lompo M, Dubois J, Guissou I. DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. International Journal of Toxicological and Pharmacological Research. 2016;8(1):29-34.

18. Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995;28(1):25-30.

19. Baky MH, Gabr NM, Shawky EM, Elgindi MR, Mekky RH. A Rare Triterpenoidal Saponin Isolated and Identified from *Tetraena simplex* (L.)

Beier &Thulin (Syn. *Zygophyllum simplex* L.). ChemistrySelect. 2020;5(6):1907-11.

20. Fan G-J, He Z-S. Triterpenoid glycosides from *Adina rubella*. Phytochemistry. 1997;44(6):1139-43.

21. Lamidi M, Ollivier E, Faure R, Debrauwer L, Nze-Ekekang L, Balansard G. Quinovic acid glycosides from *Nauclea diderrichii*. Phytochemistry. 1995;38(1):209-12.