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Research Article

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Chemical Constituents from the leaves of the Cameroonian *Jatropha gossypiifolia* Linn. (Euphorbiaceae) and biological activities of its Crude extract

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Abstract Five triterpenes : 3- Acetyl aleuritolic acid (**1**), α-Amyrin (**2**), β-Amirin (**3**), Lupeol (**6**) Taraxasterol (**7**), two polysterols : β-Sitosterol (**4**), Stigmasterol (**5**), five flavonoids : Vitexin (**8**), Isovitexin (**9**), Apigenin (**10**) Luteolin (**11**), Catechin (**12**), and three Coumarins: Tomentin (**13**), Scopoletin (**14**) and Fraxetin (**15**) were isolated from the methanolic extract of the leaves of *Jatropha gossypiifolia*. These compounds were obtained by extensive silica gel chromatography and their structures elucidated by 1D and 2D nuclear magnetic resonance (NMR) as well as comparison with literature data. Antimicrobial tests carried out on the leaf crude extract of *Jatropha gossypiifolia* showed that, bacteria *Escherichia coli* JWO451-2, and fungal *Mucor hiemalis* ATCC 20020 exhibited sensitivity to the extract.

Keywords Biological activities, Chemical composition, Euphorbiaceae, *Jatropha gossypiifolia* Linn.

Introduction

Cameroon, officially the Republic of Cameroon, is a country in central Africa. Cameroon´s rain forest counts as one of the most biologically diverse terrestrial ecosystems on earth, presenting a source of novel molecular structures and biologically active compounds. In Cameroon, as in most other developing countries, plants were used for medicinal purposes long before prehistoric period, to cure various infectious diseases caused by parasites [1-3]. Many herbal plants are widely used in traditional folk medicine of most countries worldwide [4-6]. Due to the unpleasant side effects and ineffectiveness of many conventional drugs, the search for new drugs from natural origin has gained momentum in recent years [7].

Plants have developed secondary metabolites mainly as a defense mechanism against their natural enemies [8].

The study of secondary metabolites in plants has led to the discovery of important bioactive molecules of great interest for humankind. Traditional medicine continues to be widely practised in many areas of these countries. Plants have been extensively investigated due to their biological activities and their economic value [9].

These plants are used by local medicine practitioners in various forms such as: decoctions, infusions, ointment, powder, maceration, friction and chewing. Many of the species are endemic to Western and Central [10-11].

Among these, *Jatropha gossypiifolia* L.(Euphorbiaceae), commonly known as bellyache bush, black physicnut, cotton-leaf physicnut in english, pinon negro, pinon colorado, and tua-tua in Spanish; medicinier noir and medicinier rouge in French; mamoninha and peao-roxo in Brazil; jarakmerah and sibidigua in India [12], is a shrub that grows to 2.5–4 m (8.2–13.1 ft) high (fig. 1) and contains a characteristic latex largely used for medicinal purposes, though in an empirical way. The leaves (fig. 2) are used in natural or in compresses, and are considered to have anti-malarian [13], insecticidal [14], anti-inflammatory [15] and antimicrobial [16] properties. The root and stem have cytotoxic [17], anti-malarian, leishmanicidal, antimicrobial, insecticidal, molluscicidal [18]) and anti-inflammatory [19]) properties. The seeds and fruits (fig. 3) are used against influenza, and also as laxative [18], sedative, analgesic or anti-diarrheal agents [20]. It's a species of flowering plant in the spurge family, [1] [21]. The species is native to Mexico, South America, Gujarat State (India) and the Caribbean islands. It is a declared noxious weed in Puerto Rico and is naturalised in northern Australia, including Queensland where it is listed as a Class 2 declared pest plant [21-22]. The three lobed leaves are purple and sticky when young and become bright green with age. The small red flowers with yellow centers appear in clusters. These are followed by cherry-sized seed pods that are poisonous [22]. Powdery mildew fungal disease has been reported [23].

Figure 1: J. gossypiifolia tree (by Tchamezi)

Figure 2: J. gossypiifolia fruits and flowers (by Tchamezi)

Figure 3: J. gossypiifolia leaves (by Tchamezi)

Previous chemical study of *Jatropha gossypiifolia* L. led to the isolation of classes of compounds belonging to tannins, glycosides, phenol, starch, organic acid, steroids, flavonoids, alkoloids, organic acid, saponin, diterpene, triterpene , coumarins and carbohydrate glycoside [24].

To the best of our knowledge, no phytochemical study has been done on the Cameroonian species of *Jatropha gossypiifolia*. Hence, the current work reports the isolation of fifteen known compounds from the leaves of *Jatropha gossypiifolia*. In addition, the antimicrobial and antifungal activities of the crude extract are also being reported for the first time.

Materials and Methods

Materials

Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. Infrared spectra were recorded on a JASCO 302-A spectrophotometer. EI-MS (Electronic Impact-Mass Spectra) were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluoro-kerosene as reference substance for HR-EI-MS (High Resolution-Electrospray Ionization-Mass Spectra) were measured on Agilent Techn.6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The ^{1}H- and ^{13}C-NMR spectra were recorded at 500, 400, 125 and 100 MHz, respectively on Bruker AMX 600 NMR spectrometers. Homonuclear ^{1}H connectivity was determined using the COSY (Correlation Spectroscopy) experiment. ^{1}H-^{13}C one bond connectivities were determined using HSQC (Heteronuclear Single Quantum Correlation) gradient pulse factor selection. Two and three-bonds connectivity was determined using HMBC (Heteronuclear Multiple Bond Connectivity) experiments. Chemical shifts are reported in δ (ppm) using TMS as internal standard and coupling constants (*J*) were measured in Hz. Column chromatography was carried out on silica gel (70-230 mesh, Merck). Thin Layer Chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} aluminium foil, and spots were detected using ceric sulphate spray reagent, UV lamp, iodine vapour, potassium permanganate, and vanillin. The purity of the compounds was investigated by means of TLC and LC-MS. The degree of purity of the positive control was ≥ 98%, while that of the isolated compounds was 95%. All other substances, if otherwise not specified, were purchased from Sigma-Aldrich (Germany). All reagents used were of analytical grade.

Methods

Collection and Identification of Plant Materials

The leaves of *Jatropha gossypiifolia* were collected in Yabassi, more precisely at PK25 (Littoral region of Cameroon), in November 2019. With the geographical location 4°27′16″ North, 9°57′56″ East, at an altitude of 50 m. The plant was identified by Mr. Victor Nana of the National herbarium of Cameroon, where a voucher specimen (N°60066 HNC and 25715/SFR/CAM) has been deposited.

Extraction of *Jatropha gossypiifolia* Leaves

The air-dried and powdered leaves of *Jatropha gossypiifolia* (2.5 kg), was successively extracted with methanol at room temperature for 48h and then concentrated under pressure to yield dark solid extract (180.31 g, 7.2%). Approximatively100.00 g of methanolic crude extract was subjected to silica gel column chromatography (CC) over silica gel, the elution was carried out with a mixture of "*n*-hexane-EtOAc (2/5)", "*n*-hexane-EtOAc (3/5)", "*n*-hexane-EtOAc (4/5)", "*n*-hexane-EtOAc (1/1)" in increasing polarity resulting in 4 major fractions A-D.

Fraction A (13.56 g) was composed of sub-fractions 1-52 and eluted with an isocratic system of "*n*-hexane/ EtOAc" (90/10, v/v), fractions of 100 mL were collected and treated to yield a mixture of Stigmasterol (5) and β-Sitosterol (4) (50.50 mg), α-Amirin (2) (15.25 mg), β-Amirin (3) (05.30 mg), Taraxasterol (7) (08.50 mg) and Lupeol (6) (10.50 mg). Fraction B (11.50 g) was composed of sub-fractions 53-119 and eluted with *n*-hexane-EtOAc (80/20, v/v), fractions of 100 mL were collected leading to Acetyl aleuritolic acid (1) (15.50 mg) and Tomentin (13) (12.55 mg). Fraction C (25.50 g) resulting from sub-fractions 120-192 was eluted with *n*-hexane-EtOAc (50/50), fractions of 100 mL were collected to give Scopoletin (14) (13.50 mg) and Fraxetin (15) (08.50 mg). Finally, fraction D (20.25 g) was composed of sub-fractions 193-251 followed by elution with *n*-hexane-EtOAc (20/80, v/v, up to 100% EtOAc) and fractions of 100 mL were collected to afford, Apigenin (10) (15.50 mg), Luteolin (11) (09.50), Catechin (12) (07.50 mg), Vitexin (8) (5.50 mg) and Isovitexin (9) (18.50mg).

Antibacterial and antifungal assays

The *Jatropha gossypiifolia* extract was tested on the two bacterias strains namely Bacillus subtilis DSM10 (Gram-positive), *Escherichia coli* BW25113 and *Escherichia coli* JWO451-2 (Gram-negative), as well as one filamentous fungi such as *Mucor hiemalis* ATCC 20020.

Preparation of bacterial and fungal inoculum

The fungal strain was freshly cultured on Sabouraud dextrose agar plate in 90 mm Petri dish and incubated at 27° C for seven days. After incubation, spore fungal were removed and suspended in 2 ml of sterile distilled water. The fungal suspensions were filtered once through a sterile gauze to remove hyphae**.** The resulting solution was adjusted at 10^5 spores/mL by using a hemacytometer cell.

For bacterial inoculum, the pre-culture was done on Muller-Hinton agar plates and incubated at 37° C for 24h. A bacterial colony of these each pre-culture taken separately was introduced in test tube containing 6 ml of sterile distilled water. Bacterial suspension was adjusted to McFarland standard 0.5.

Broth microdilution test

The antifungal and antibacterial activities of *Jatropha gossypiifolia* extracts were evaluated using the Broth microdilution method in 96-well microplates (NUNCTM) as described by Rampadarath [25]. In fact, 100 µL of the broth of Mueller-Hinton were distributed in the wells of the microplate. Then, 100 µL of extract solution at a concentration of 4096 µg/mL were introduced into the upper wells. It was followed by a series of 7 dilutions of ratio 2 whose concentration of first microcupule was 1024 and of the last 4 µg/mL. In control well, the extract was replaced for methanol. Finally, the content of each well (100 µL) was diluted by adding 100 µL of bacterial or fungal inoculum. The microplates thus treated were incubated at 37° C for 24h for bacteria test and 27 ° C for 48h for filamentous fungi test.

The sensitivity of microorganisms to the extract is observed when the microcupule was not exhibit turbidity (in case of bacteria) or when the well was not presented the visible growth of fungal. The minimum inhibitory concentration was considered to be the smallest concentration which would prevent the formation of turbidity or growth of microorganisms in well.

Results and Discussion

The methanol extract of the air-dried leaves of *Jatropha gossypiifolia* was chromatographed on a column of silica gel eluted with pure *n*-hexane, then using "*n*-hexane-EtOAc" mixture and finally EtOAc in increasing polarity to afford fifteen known compounds (Fig.4). By comparison with the reported data, the known compounds were identified as : Acetyl aleuritolic acid (1) [27], α-Amyrin (2) [28], β-Amyrin (3) [28],a mixture of β-sitosterol (4) and stigmasterol (5) [29], lupeol (6) [30], taraxasterol (7) [31], Vitexin (8) [32], Isovitexin (9) [33], Apigenin (10) [34], Luteolin (11) [35], Catechin (12) [36], Tomentin (13) [37], Scopoletin (14) [38], Fraxetin (15). [37] (Fig.4).

Antimicrobial tests carried out on the extract of *Jatropha gossypiifolia* leaves, showed that, bacteria *Escherichia coli* JWO451-2 and fungal *Mucor hiemalis* ATCC 20020 exhibited sensitivity to the extract. Indeed, it was observed only in the upper well containing these microorganisms the absence of turbidity or visible growth of microorganism. The concentrations of extracts in these wells (1024 µg/Ml) would be considered like MIC. These results correlate with tests previously carried out by Rampadarath [25] and Viswanathan [26] who showed the antibacterial activity of *Jatropha gossypiifolia* extract against E. coli strains. This lack of turbidity or visible growth of *Escherichia coli* and *Mucor hiemalis* would be due to the ability of the extract to inhibit the multiplication of microorganisms. This antibacterial and antifungal activity could be attributed to diterpenes, coumarins as well as the flavonoids and cyclic peptides present in plants of the genus.

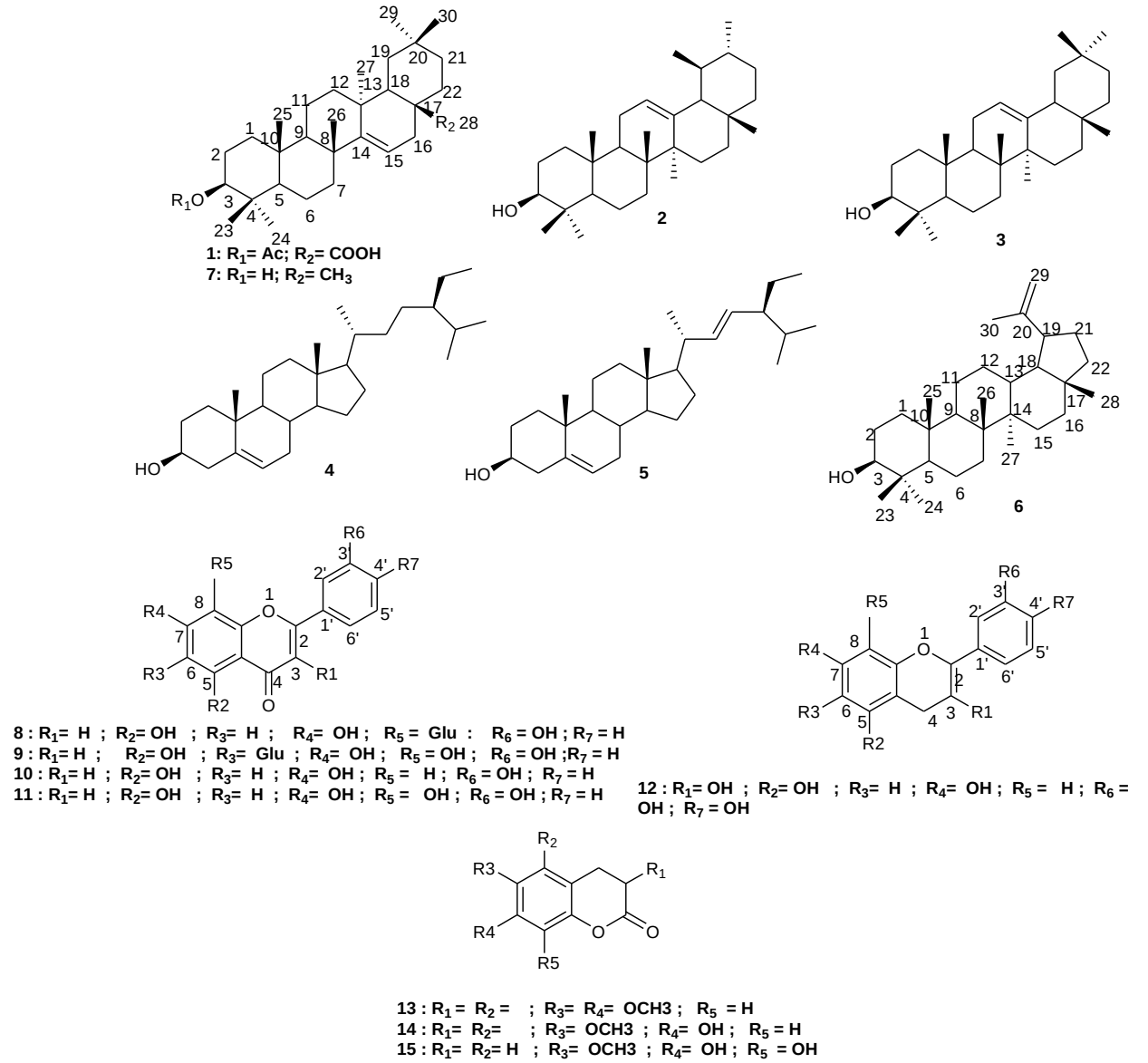

Figure 4: Structures of compounds (1 – 15) isolated and characterized from the leaves of Jatropha gossypiifolia

Conclusion

This study shows that the methanolic crude extract of the leaves of *Jatropha gossypiifolia* **(**are rich in phytochemicals such as triterpenes, flavonoids, polysterols and coumarins which are of anti-infective importance. In the present work, we report the isolation of fifteen previously described compounds: five triterpenes (1-3, 6-7), two polysterols (4-5), five flavonoids (8-12), and three coumarins (13-15). The antimicrobial effects of the methanolic leaves crude extract of *Jatropha gossypiifolia* were evaluated against three bacteria: Bacillus subtilis DSM10 (Gram positive), *Escherichia coli* BW25113 and *Escherichia coli* JWO451-2 (Gram negative), as well as filamentous fungi such as *Mucor hiemalis* ATCC 20020. According to the reference [25] we can say that *Escherichia coli* JWO451-2 (Gram negative) and *Mucor hiemalis* ATCC 20020 exhibited sensitivity to the methanolic crude extract.

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References

[1]. Pavunraj, M., Ramasubbu, G., Baskar, K., (2017). *Leucas aspera* (Willd.) L. Antibacterial, antifungal and mosquitocidal activities. Trends Phytochem. Res. 1, 135-142.

[2]. Mohammadhosseini, M., (2017). The ethnobotanical, phytochemical and pharmacological properties and medicinal applications essential oils and extracts if different *Ziziphora*species. Ind. Crops Prod. 105(1), 164-192.

[3]. Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A. (2017). Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199, 257-315.

[4]. Frezza, C., Venditti, A., Serafini, I., Carassiti, A., Foddai, S., Bianco, A., Serafini, M. (2017). Phytochemical characteristics of *Galeopsis ladanum* subsp. *Angustifolia* (Ehrh. ex Hoffm.) Gaudin collected in Abruzzo region (Central Italy) with chemotaxonomic and ethnopharmacological implications. Trends Phytochem. Res. 1(2), 61-68.

[5]. Venditti, A., Bianco, A. (2018). Secondary metabolites of *Hypericum richer* Vill. Collected in Central Italy: chemotaxonomy and ethnomedicinal relevance. Trends Phytochem. Res. 2(3), 155-162

[6]. Venditti, A., Frezza, C., Serafini, I., Pulone, S., Scardelletti, G., Sciubba, F., Bianco, A., Serafini, M. (2018). Chemical profiling of the fruits of *Styrax officinalis* L. from Monti Lucretili (Latium region, Central Italy): Chemotaxonomy and nutraceutical potential. Trends Phytochem. Res. 2(1), 1-12.

[7]. Mohammadhosseini, M., Venditti, A., Sarker, S.D.,Nahar, L., Akbarzadeh, A. (2019). The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities – A review. Ind. Crop Prod. 129 (2019) 350-394.

[8]. Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64, 3-9.

[9]. Mohammadhosseinia, M., Satyajit, D., Sarkerb, A. A. (2016). Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. Journal of Ethnopharmacology, 199, 255-315 doi.org/10.1016/j.jep.2017.02.010.

[10]. Wansi, J.D., Sewald, N., Lutfun, N., Claire, M., Satyajit, D.S. (2018). Bioactive essential oils from the Cameroonian rain forest: A review- Part I. Trends Phytochem. Res. 2(4), 187-234.

[11]. Wansi, J. D., Sewald, N., Lutfun, N., Claire, M., Satyajit, D.S. (2019). Bioactive essential oils from the Cameroonian rain forest: A review- Part II. Trends Phytochem. Res. 3(2), 3-52.

[12]. Félix-Silva, J., Giordani, R.B., Silva-Jr, A.A.da., Zucolotto, S.M., Fernandes-Pedrosa, M.de F. (2014). "Jatropha gossypiifolia L. (Euphorbiaceae): A Review of Traditional Uses, Phytochemistry, Pharmacology, and Toxicology of This Medicinal Plant". *Evidence-Based Complementary and Alternative Medicine. 369204.* Doi*:* 10.1155/2014/369204.

[13]. Jansen. O., Angenot. L., Tits, M., Nicolas, J.P., Mol, D.P., Nikiema, J.B., & Frederich, M. (2010). Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. J Ethnopharmacol 130, 143-150.

[14]. Valencia, A., Frérot, B., Guénego, H., Múnera, D.F., Sá, M.F.G.D., & Calatayud, P.A. (2006). Effect of Jatropha gossypiifolia leaf extracts on three *lepidoptera* species. Rev Colomb Entomol 32, 45-48.

[15]. Oliveira, F.C.S., Barros, R.F.M., Neto, M.J.M. (2010). Plantas medicinais utilizadas em comunidades rurais de Oeiras, semiárido piauiense. Ver Bras Pl Med. 12, 282-301.

[16]. Gaikwad, R.S., Kakde, R.B., Kulkarni, A.U., Gaikwad, D.R., Panchal, V.H. (2012). *In vitro* antimicrobial activity of crude extracts of *Jatropha* species. Curr. Bot. 3, 09-15.

[17]. Nazeema, T.H., & Girija, S. (2013). Characterisation of the active antiproliferative principles of *Jatropha curcus* and *Jatropha gossippifolia* on Hela cell lines. Int. J. Pharm Pharm Sci 5, 346-355.

[18]. Sabandar, C.W., Ahmat, N., Jaafar, F.M., & Sahidin, I. (2013). Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): A review. Phytochemistry 85, 7-29.

[19]. Bhagat, R., Misar, A.V., Ambavade, S.D., & Kulkarni, D.K. (2013). HPTLC analysis and anti-inflammatory activity of *Jatropha gossypifolia* L. Root in mice and wistar rats. IJPR 3, 13-17.

[20]. Apu, A.S., Hossain, F., Rizwan, F., Bhuyan, S.H., Matin, M., & Jamaluddin, A.T.M. (2013). Study of pharmacological activities of methanol extract of *Jatropha gossypifolia* fruits. J. Basic ClinPharm 4, 20-24.

[21]. "Jatropha gossypiifolia". Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Retrieved 7 June 2012.

[22]. "Bellyache bush". Agriculture, Fisheries and Forestry (Queensland Government. Archived from the original on 12 November 2012. Retrieved 7 June 2012.

[23]. Reddy, J.R., Reddy, A.P., (1980). "Erysiphe euphorbiae sp. nov. On Jatropha gossypifolia L". Current Microbiology. 4 (2), 95. Doi:10.1007/BF02602900.

[24]. Saini, V., Mishra, R., Mandloi, S., Yadav, N. (2015). Analysis of phytochemical content of *Jatropha gossypiifolia* L. Chemical and Process Engineering Research V.35.

[25]. Rampadarath, S., Daneshwar, P., Rajesh, J. (2016). *Jatropha curcas* L. Phytochemical, antimicrobial and larvicidal properties. Asian Pacific Journal of Tropical Biomedicine. 6(10), 858-865. doi.org/10.1016/j.apjtb.2016.01.019.

[26]. Viswanathan , L., Carol, E., Golin, C.D., Jones, M., Ashok, S., Blalock., Roberta, C. M., Wines, E.J., Coker-Schwimmer, L., Rosen, D.L., Priyanka, S., Lohr., K.N. (2012). Interventions to improve adherence to self-administered medications for chronic diseases in the United States: a systematic review. Annals of international medicine. doi.org/10.7326/0003-4819-157-11-201212040-00538

[27]. Rosmawati, A.A., Norizan, A. (2016). Isolation of acetyl aleuritolic acid from *Pimeleodendron griffithianum* (Euphorbiaceae). Malaysian Journal of Analytical Sciences, 20(3), 573–577. DOI.org/10.17576/mjas-2016-2003-16

[28]. Cardoso, K. B., De Oliveira, M. L.H., Melo, Z. U., Fernandez, M.C.M., De Araújo, F.A.C.C., Gonçalves, J.E., Laverde, A.Jr., Romagnolo, B. M., Linde, A.G., & Gazim, C.Z. (2018). Antioxidant activity of α and β-amyrin isolated from *Myrcianthes pungens* leaves, Natural Product Research, DOI: 10.1080/14786419.2018.1525715

[29]. Prakash, C.V.S., Zhijie, G., Sidney, M., Hecht, S.H., Jones, D.G.I., Kingston, (2003). A New Acylated Oleanane Triterpenoid from *Couepia polyandra* that inhibits the lyase activity of DNA polymerase. J. Nat. Prod. 66(11), 1463-1465. DOI: org/10.1021/np0301893.

[30]. Ekalu, A., Gbekele-oluwa ayo, R. , Habila, J., Hamisu, İ. (2019). Bioactivity of Phaeophytin a, α-Amyrin and lupeol from *Brachystelma togoense* Schltr. Journal of the Turkish Chemical Society Section A: Chemistry Volume 6(3), 411 – 418. doi.org/10.18596/jotcsa.571770

[31]. Aduku, O.G., Abdullahi, S.M., Sule, M.I., Atiku, I., Anyam, J.V. (2020). Isolation of Taraxasterol and Stigmasterol from the zerial part of *Centaurea perrottetii* Dc. (Asteraceae). Bima Journal of Science and Technology, 4(2). 86-93.

[32]. Muhammad, S., Muhammad, A., Syarhabil, A., Mamat, A.So. (2015). Pharmacological potential of Vitexin. Indian Research Journal of Pharmacy and Science.

[33]. Lee, J.S., Lee, A.Y., Quilantang, N.G., Geraldino, P.J.L., Eun, J.C., Lee, S. (2019). Anti-oxidant activity of avicularin and isovitexin from *Lespedeza cuneata*. J Appl Biol Chem, 62(2), 143−147 doi.org/10.3839/jabc.2019.020.

[34]. Liu, H.C., Jin, Y.S., Chenhai, S. (2013). Study on triterpenoid and flavonoid contents of *Rabdosia japonica*. . Acad. J. *Second* Mil. Med. Univ., 34(10), 1121-1124.

[35]. Esposito , F., Sanna, C., Del Vecchio, C., Cannas, V., Venditti, A., Corona, A., Bianco, A., Serrilli, A.M., Guarcini, L., Parolin, C., Ballero, M., Tramontano, E., 2013. *Hypericum hircinum* L. components as new single-molecule inhibitors of both HIV-1 reverse transcriptase-associated DNA polymerase and ribonuclease H activities. Pathogens and disease 68(3). 116-24. Doi 10.1111/2049-632X.12051.

[36]. Bae, J., Kim,N., Shin, Y., Kim, S.Y., & Kim, Y.J. (2020) Activity of catechins and their applications. Biomedical Dermatology 4:8 Doi.org/10.1186/s41702-020-0057-8

[37]. Truong, N. M., Tran, D. X., Tran H.D., Truong, M.V., Yusuf, A., Tran, D.K., Nguyen, V.Q., Ahmad, A. (2019). Isolation and Purification of Bioactive Compounds from the Stem Bark of Jatropha podagrica Molecules 24, 889. Doi: 10.3390/molecules24050889

[38]. Huda, A. R., Kunert, F.O., Haslinger, E., & Seger, C. (2002). Isolation and Structure Elucidation of Iridoide and Coumarin Derivatives from *Xeromphis nilotica* (Rubiaceae) Monatshefte fur Chemie 133, 1453–1458 DOI 10.1007/s00706-002-0500-0