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Phytochemical Studies and GC-MS Analysis of Chloroform Extract of the Leaves of *Aspilia africana*

Ogbemudia Ruth Etiosa[1*], Adeyemo John Akeem[1] and Nwadozie Blessing Chika[2]

[1]University of Benin, Benin-City, Edo State, Nigeria.
[2]College of Education, Agbor, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ORE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ORE and AJA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: The aim of this research was extraction, phytochemical studies and GCMS analysis of *Aspilia africana*.
Place and Duration of Study: This study was carried out at the University of Benin, Benin-City and University of Lagos Central Research Laboratory, Nigeria from January 2016 to September 2016.
Methodology: 200 g of the pulverized plant sample was extracted with hexane, chloroform and methanol in this order for 8-12 hours. The extract was concentrated using a rotary evaporator to obtain the crude extract. The phytochemical test was carried out using standard methods. The GCMS was carried out using Agilent Technologies 7890A couple with Agilent Technologies 5975C VL MSD. The mobile phase is helium gas while the stationary phase was the column agilent technology HP5 MS with length 30m, internal diameter 0.320 mm and thickness 0.25 microns. The volume injected is 1 microlitre, oven initial temperature was 80°C to hold for 2 minutes. The mode was split less and scan range was 35-55.

**Corresponding author: E-mail: ruchino898@yahoo.com;*

Results: The phytochemicals of the leaves of *Aspilia africana* were extracted with chloroform by Soxhlet method and analysed using GC-MS. Ten constituents were identified constituting 92.714% hydrocarbon. Caryophyllene (29.43%), 2-Carene (19.76%) and Germacrene D (33.3%) were the major components of the extract. The phytochemical analysis was carried out by standard methods. The results revealed the presence of a number of therapeutically important phytochemicals such as alkaloids, terpenoids, tannins, flavonoids, saponins and glycosides in the extract.

Conclusion: The results of the phytochemical screening and GC-MS analysis indicate that the leaves of *Aspilia africana* are a rich source of a number of bioactive secondary metabolites which include alkaloids, tannins, saponins, flavonoid, glycosides and terpenoids. This justifies the use of the plant in the traditional practices of herbal medicine, especially in the treatment of microbe-induced disease conditions.

Keywords: Phytochemical; Aspilia africana; germacrene D; caryophyllene; terpenoids; analgesic.

1. INTRODUCTION

Aspillia africana C.D. Adams (*Asteraceae*) is a tropical shrub widely distributed across tropical Africa. In Nigeria, it is commonly known as yurinyun by the Yorubas, Orangina by Igbos, Tozalin by Hausas and Edemedong by Efiks [1]. *Aspilia africana* is of high economic and medicinal importance due to its active roles in wound healing, treatment of rheumatic pains etc. *A. africana* is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores, in the treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies from the eyes [2]. It is commonly used to feed livestock particularly sheep and goats. It is believed to have nutritive and medicinal values. Many people and livestock are suffering from malnutrition and diseases due to deficiencies of major food groups such as proteins, fats, carbohydrates and vitamins. The effect of malnutrition includes poor growth rate, decreased resistance to diseases, mental retardation, and lethargy in extreme cases starvation and death may occur [2]. The plant *Aspilia africana* amongst other weed was reported to be a source of protein although the quantity is not sufficient for both human and livestock demands [3]. Moreover, *Aspilia africana* is used locally to treat ear infections and stomach ailments. The phytochemistry of any plant part is concerned with the enormous variety of organic substances that are accumulated by the plant, and also brings in focus the chemical structures of these substances. [4,5] investigated the presence of fatty acid in the oil of seeds of *A. africana* and the presence of diterpenes, kaurenoic and grandiflorenic acids from the leaves.

2. MATERIALS AND METHODS

Analytical reagents (AR) solvents were used.

2.1 Collection of Plant Materials

The leaves of *Aspilia africana* were collected from the University of Benin, Benin-City, Edo State and identified by Professor Bamidele of the Department of Botany, University of Benin, Benin-City, Nigeria.

2.2 Methods of Extraction

The leaves were air dried at room temperature of 25±2°C for 3 weeks under a shade on the side bench in the laboratory to prevent the loss of volatile organic components of the leaves that may result from the direct effect of sunlight. The dried leaves of *A. africana* were milled into coarse powder by a mechanical grinder and 100 g of the pulverized, air dried leaves were extracted with chloroform by soxhlet extraction for 4– 6 hours. The extract was concentrated to obtain crude chloroform extract and then stored at 4°C until required for use.

2.3 Phytochemical Screening of the Leaf Extract of *A. africana*

The preliminary phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, anthraquinones, tannins, terpenes was carried out using standard procedures [6,7].

2.4 GC-MS Analysis of *Aspilia africana* Chloroform Leaf Extract

The analysis of the crude was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass selective detector (VLMSD, Agilent 5975C) equipped with a non-polar Agilent HP-5MS (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.32 mm i.d. and 0.25 µm film thickness) with Injector series (Agilent, 7683B). The carrier gas was helium with linear velocity of

1 ml/min. Oven temperature was set at 80°C for 2 minutes, then programmed until 120°C at the rate of 5°C/min withhold time of 2 minutes, and finally increased to 240°C at the 10°C/min rate, isothermal at the temperature for 6 min hold time. Injector and detector temperatures were 300°C and 200°C respectively. Injection mode, splitless, volume injected, 1 µl of the oil. The MS operating parameters were as follows: Ionization potential, 70 eV; interface temperature, 200°C and acquisition mass range; 50-800. Relative percentage amounts of the essential oil components were evaluated from the total peak area (TIC) by apparatus software.

2.4.1 Identification of components

Identification of components in the extract was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.5 Proximate Analysis

After bringing the samples to uniform size, they were analysed for moisture, protein, fat, ash, fibre and nitrogen free extract by the methods of AOAC [8] and conducted in Ph. D laboratory of the Department of Chemistry, University of Benin, Benin city, Nigeria.

2.5.1 Determination of moisture

Moisture was determined by oven drying method. 1.0 g of well-mixed sample was accurately weighed in clean, dried crucible (W_0). The crucible was allowed in an oven at 100-105°C for 6-12 h until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling, it was weighed again (W_1). The percent moisture 2 was calculated by following formula:

$$\% \text{ Moisture} = \frac{W_o - W_1}{W_o} \times 100$$

Where:

W_o = Initial weight of Crucible + Sample
W_1 =Final weight of Crucible +Sample
Moisture free sample was used for further analysis

2.5.2 Determination of ash

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1). One gram of each of sample was taken in crucible (W_2). The sample was ignited over a burner with the help of blowpipe, until it was charred. Then the crucible was placed in muffle furnace at 550°C for 2-4 h. The appearance of gray white ash indicated complete oxidation of all organic matter in the sample. After ashing, furnace was switched off. The crucible was cooled and weighed (W_3). Percent ash was calculated by the following formula:

$$\% \text{ Ash} = \frac{\text{Difference in weight of Ash}}{\text{Weight of sample}} \times 100$$

Difference in wt. of Ash= $W_3 - W_1$

2.5.3 Determination of crude fiber

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute (0.128 M, 7.1 ml in 992.9 ml distilled H_2O) H_2SO_4 and then with dilute (0.223 M, 12.5 g in 1 dm^3) KOH solution. The undigested residue collected after digestion was ignited and loss in weight after the ignition was registered as crude fiber.

1.0 g sample (W_0) was weighed and transferred into a porous crucible. The crucible was placed into Dosi-fiber unit and the valve was kept in "OFF" position. 150 ml of preheated H_2SO_4 solution and some drops of foam-suppresser (acetone) was added to each column. Then the cooling circuit was opened and the heating elements (power at 90%) were turned on. As the mixture started boiling, the power was reduced to 30% and left for 30 minutes. The valves were opened for drainage of acid and were rinsed with distilled water three times to completely ensure the removal of acid from the sample. The same procedure was used for alkali digestion using KOH instead of H_2SO_4. The sample was dried in an oven at 150°C for 1 hour. Then the sample was allowed to cool in a desiccator and weighed (W_1). The crucible was transferred into the muffle furnace at 550°C for 3-4 hours and then, cooled in a desiccator and weighed again (W_2).

Calculations were done by using the formula:

$$\%\text{Crude Fiber} = \frac{W_1 - W_2}{W_0} X\ 100$$

2.5.4 Determination of crude fat

Dry extraction method for fat determination was implied. It consisted of extracting dry sample with

some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. were extracted together, therefore, the results are frequently referred to as crude fat. Crude fat was determined by ether extract method using Soxhlet apparatus.

1 g of the moisture-free sample was weighed and wrapped in a filter paper, placed in a fat-free thimble and then introduced into the soxhlet. Weighed, cleaned and dried receiving round bottom flask was three quarter filled with petroleum ether and fitted to the soxhlet. A condenser was fitted into the soxhlet with water and heater turned on to start the extraction. After 4–6 c, and the ether was allowed to evaporate and then the flask was disconnected before the last siphoning. The extract was transferred into a clean glass dish rinsed with ether then, the ether was evaporated on water bath. Then the dish was placed in an oven at 105°C for 2 hours and cooled in a dessicator. The percent crude fat was determined by using the following formula:

$$\%\text{Crude Fat} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

2.5.5 Determination of crude protein

Crude protein was determined by measuring the nitrogen content of the sample and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen.

Crude protein was determined by the modified Kjeldahl method as reported by Ikpe and Akpabio [9]. The method involved digestion, distillation and titration.

2.5.5.1 Digestion

1 g of the sample was weighed into the Kjeldahl flask; well clamped to a retort stand under a regulated hot plate inside the fume cupboard. The mixed acid of 10 ml HNO_3 and HCl was introduced in the ratio of 1:3 respectively. The flask was heated slowly, later increased until one-third of the digest remained. The digest was diluted with distilled water and later filtered and made-up to mark of 100 ml standard volumetric flask.

2.5.5.2 Distillation

500 ml round bottom flask was fixed to a heating unit. 10 ml aliquot of the digest was added to the flask. The aliquot digest was diluted with 50 ml distilled water. 40% NaOH was added to give an alkaline medium then, the condenser was mounted and connected to the receiver (250 ml conical flask). 50 ml of 4% boric acid and a double indicator (methyl red and Bromo cresol in ratio 4:1) was added to the receiver. The regulated heating mantle was switched on at low temperature and later increased. The distillate was collected for titration.

2.5.5.3 Titration

The biurette was filled with 0.1N HCl and titrated against the distillate. The colour changed from green to light pink at the end point. This was repeated twice. The blank was also titrated and the titre value was deducted from the real titre in order to have the actual value.

$$\%N = \frac{(S-B)\ X\ \ M\ X\ 14\ X\ D}{W\ x\ 1000\ x\ V} x\ 100$$

Where, S = Average titre value, B = Blank titre value, M = Molarity or Normality of HCl, 14 = Atomic weight of nitrogen, D = Total volume of digest, W = Weight of sample, 1000 = milli equivalent of nitrogen, V = Volume taken for distillation.

Crude protein was calculated as: 6.25 x %N.

2.5.6 Determination of nitrogen-free extract

Nitrogen Free Extract (NFE) was determined by mathematical calculation. It was obtained by subtracting the sum of percentages of all the nutrients already determined in proximate analysis from 100.

NFE = (100-% moisture + % crude protein + % crude fat + % crude fiber + % ash)

2.5.7 Energy calculation

The percent calorie was calculated by multiplying the percentage of crude protein and carbohydrate by 4 and crude fat by 9. The values were then added together as calories per 100 g of the sample.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of the Leaf Extract of *Aspilia africana*

The result of the phytochemical screening of the leaf extract of *Aspilia africana* presented in

Table 2 showed the presence of alkaloids, tannins, saponins, flavonoids, eugenols, glycosides and terpenoids. Majority of these phytochemicals have been reported to be high of therapeutic value [10,11]. The presence of flavonoids justifies the traditional uses of the plant as a pain reliever and for its anti-inflammatory activities. Presence of tannins and saponins in the leaf extract agrees with the work of Obadoni and Ochuko [12] who reported tannins and saponins as the most abundant constituents of the plant. One or a combination of these phytoconstituents may be responsible for its analgesic and anti-inflammatory activities. Saponins and related phytosterols [13]; alkaloids [13,14]; phenolics and flavonoids [13,15,16]; and some glycosides [17] have been reported to possess antinociceptive and/or anti-inflammatory activities. Oxidative stress has been reported to be among the major causative factors of many degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [18]. Phytochemicals such as tannins and flavonoids, which are present in *A. africana* as revealed by the results of the phytochemical screening, have been reported to display antioxidant activity [18]. They, therefore, prevent oxidative cell damage and protect against allergies, inflammations and a host of degenerative diseases [19,20,21].

Tannins and saponins regulate, protect and kill cancer cells such as cancer of prostrate, testicular cancer and improve semen quality in men [22]. They also prevent breast cancer and cystic ovaries in women. Alkaloids are analgesics, antispasmotic for the treatment of hypertension, mental disorders and for pupil dilation [20,21]. The presence of these bioactive compounds as revealed by the phytochemical screening of the chloroform extract of the leaves of *A. africana*, therefore, provides the scientific backings and validates the use of the plant in the practices of herbal medicine in Nigeria.

Table 1. Phytochemical test of crude chloroform extract of *A. africana*

Phytochemicals	Leaf	Flower
Glycosides	+	+
Saponins	+	+
Flavonoids	+	+
Phenolics	-	+
Tannins	+	+
Eugenols	+	+
Steroids	-	-
Terpenoids	+	+
Alkaloids	+	+

3.2 GC-MS Analysis of *A. africana*

Three constituents were predominantly found in the leaves of *A. africana* and are hydrocarbon compounds. They include 2-Carene (19.76%), Caryophyllene (29.43%) and Germacrene D (33.3%). These constituents have been found to show interesting biological activity against certain illness and/or pathogens. (E)-Caryophyllene is known for its anti-inflammatory and local anaesthetic properties [6]. Germacrene D and

Table 2. Phyto-constituents identified from *A. africana* by GC-MS analysis

Peak#	RT	Area%	Qual.	Compounds	Molecular formula	Molecular mass
1	9.141	19.76	93	2-carene	$C_{10}H_{16}$	136
2	10.096	1.36	38	2,4-Dimethyl-3-nitrobicyclo[3.2.1] octan-8-one	$C_{10}H_{15}NO_3$	197
3	10.565	29.43	90	Caryophyllene	$C_{15}H_{24}$	204
4	11.138	1.63	45	Bicyclo[2.2.1]heptane,7,7-dimethyl-2-methylene	$C_{10}H_{16}$	136
5	11.578	33.30	96	Germacrene D	$C_{15}H_{28}$	208
6	11.801	2.73	43	1,5-Heptadiene,2,5-dimethyl-3-methylene-	$C_{10}H_{16}$	136
7	12.173	3.66	38	3-Methylbenzylalcohol	$C_8H_{10}O$	122
8	18.370	2.85	39	Trans-1,3-diisopropenyl-Cyclobutane	$C_{10}H_{16}$	136
9	19.234	2.27	40	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane trans	$C_{15}H_{26}O$	222
10	20.264	1.70	38	5-Ethyl-2,2,3-trimethyl Heptane	$C_{12}H_{26}$	170

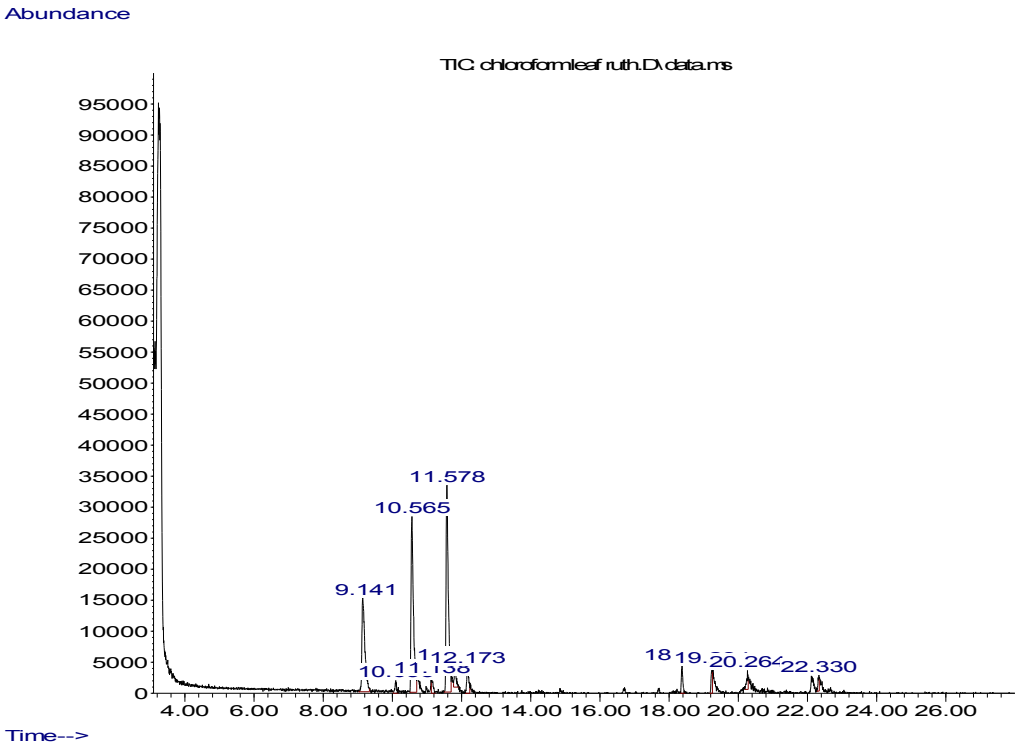

Fig. 1. Gas chromatogram of the chloroform crude extract of *A. africana*

Caryophyllene have significant antibacterial and antifungal activities [19]. Carene is a bicyclic monoterpene which occurs naturally as a constituent of turpentine. A study has shown that carene provides anti-inflammatory effects in mice [20]. The activities shown by these compounds are consistent with the herbal traditional practices and ethnobotanical uses of *Aspilia africana* in many parts of Nigeria.

3.3 Proximate Analysis

The results of the proximate analysis of the leaves of *A. africana* are presented in Table 3. The results are fairly in agreement with those reported by other authors [1,23]. The lower moisture content (10.58±0.15%) as compared with the standard value (14%) reported by African Pharmacopoeia [23] and (20%) by Ibrahim, Ajaegbu and Egharevba [24] indicates that the crude plant can be stored for a longer period of time with lower chances of microbial attack and growth which may decompose the important secondary metabolites of the plant. Total ash component (14.80 ± 0.02%) also indicates the plant has a fair share of the inorganic components in addition to its numerous bioactive constituents. The results of other components of the proximate analysis (Protein (3.94 ± 0.01%), Crude fibre (8.40±0.01%) and carbohydrate (41.31±0.02%)) have also revealed a measure of the plant's nutritional value in addition to its known medicinal relevance in Nigeria.

Table 3. Proximate analysis of *Aspilia africana* plant

Composition	Leaf value (%)	Flower value (%)
Protein	3.94 ± 0.01	5.69 ± 0.12
Crude fat/oil	20.97 ± 0.01	19.96 ± 0.01
Ash	14.80 ± 0.02	9.70±0.01
Moisture	10.58 ±0.15	9.80±0.11
Crude fibre	8.40±0.01	25.60±0.01
Carbohydrate	41.31±0.02	29.25±0.01
Energy	369.73 ± 0.00 kcal/100 g	319.40±0.01 kcal/100 g

4. CONCLUSION

The result of the phytochemical screening indicates that *Aspilia africana* is a rich source of a number of bioactive secondary metabolites which include alkaloids, tannins, saponins, flavonoid, glycosides and terpenoids. This justifies the use of the plant in the traditional practices of herbal medicine, especially in the treatment of microbe-induced disease conditions such as dysentery, wound sepsis, gonorrhea among others. However, very few pure compounds have been isolated from the plant. Further research should therefore be carried out in isolating pure compounds responsible for all these activities. Possible synthesis and derivatisation of these compounds may afford more formidable drugs in combating fungal and bacterial-related diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abii TA, Onuoha EN. The chemical constituents of the leaf of *Aspilia africana* as a scientific backing to its tradomedical potentials. Agricultural Journal. 2011;6(1): 28-30.
2. Iwu MM. Handbook of African medicinal plants. CRP Press, Boca Raton, Florida; 1993.
3. Skellern C, Rogers P. Nutrition basic botany. Academic Press, New York, London. 1977;100-105.
4. Umoh IB, Oke OL. Nutritive value of some lesser known oil seeds. Food Chemistry. 1974;2:315-321.
5. Page JE, Baiza F, Nishida T, Towers GHM. Biologically active diterpenes from *Aspilia mossambicensis.* Chimpanzee Medicinal Plant Phytochemicals. 1992; 31(10):3437-3439.
6. Trease GE, Evans WC. Pharmacognosy, 15th Edition, W.B Sounders Limited, London. 2002;585.
7. Harbone JD. Phytochemical methods, 2nd Ed. Chapman and Hall, London. 1998;60, 69,87,94.
8. AOAC International. Association of Official Analytical Chemists International. Official methods of analysis. 15th Edition; Gaithersburg, MD; 2003.
9. Ikpe EE, Akpabio UD. Proximate composition and nutrient analysis of *Aneilema aequinoctiale* leaves. Asian Journal of Plant Science and Research. 2013;3(2):55-61.
10. Buricova L, Reblova Z. Czech medicinal plants as possible sources of antioxidant effects. Czech Journal of Food Science. 2008;28(2):132-138.
11. De N, James NA. Antibacterial spectrum of extracts of *Ocimum gratissium*. Nigerian Journal of Applied Science. 2002;11:165-175.
12. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences. 2002;8. (Abstract)
13. Larkins N, Wynn S. Pharmacognosy; phytomedicines and their mechanisms. Veterinary Clinic Small Animal Practice. 2004;34:291-327.
14. Raaman N. Categories of phytochemicals. In: Phytochemicals Techniques. New India Publishing Agency, Pitam Pura, New Delhi. 2006;197-274.
15. Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel Filho V, Yunes R. Naturally occurring antinociceptive substances from plants. Phytotherapy Research. 2000;14: 401-418.
16. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacology. 2002;2:7. Available:http://www.biomedcentral.com/1471-2210/2/7
17. Ma S, Zhou S, Shu B, Zhou J. Pharmacological studies on *Crocus glycosides* I. Effects on anti-inflammatory and immune function. Zhongcaoyao. 1998;26:536-539.
18. Young IS, Woodside JV. Antioxidants in health and disease. 2001;54:176-186.
19. Essien EE, Newby JM, Walker TM, Ogunwande IA, Setzer WN, Ekundayo O. Essential oil constituents, anticancer and antimicrobial activity of *Ficus mucoso* and

Casuarina equisetifolia leaves. American Journal of Essential Oils and Natural Products. 2016;4(1):01-06.

20. Ocete MA, Risco S, Zarzuelo A, Jimenez J. Pharmacological activity of the essential oil of *Bupleurum gibraltaricum*: Anti-inflammatory activity and effects on isolated rat uteri. Journal of Ethno-pharmacology. 1989;25(3):305-13.
21. Okwu DE, Omodamiro OO. Effect of hexane extract and phytochemical content of *Xylopia aethiopica* and *Ocimum gratissimum* on uterus of guinea pig. Bio-research. 2005;3(2):40–44.
22. Wahlqvist ML, Kouris-Blazos A, Dalais F, Kannar D, Wattanapenpaiboon N. Phytochemical deficiency disorders: Inadequate intake of protective foods. Current Therapeutics. 1998;39(7):53-6,58-60.
23. Essiett UA, Akpan EM. Proximate composition and phytochemical constituents of *Aspilia africana* (Pers) C.D Adams and *Tithonia diversifolia* (Hemsl) A. Gray stems (*Asteraceae*). Bulletin of Environmental, Pharmacology and Life Sciences. 2013;2(4):33-37.
24. Ibrahim J, Ajaegbu VC, Egharevba HO. Pharmacognostic and phytochemical analysis of *commenlina benghalensis* L. Journal of Ethnobotanical Leaflets. 2010;14:610-615.

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