



UNIVERSITI PUTRA MALAYSIA

***ETHNOBOTANICAL SURVEY AND BIOLOGICAL PROPERTIES OF
MEDICINAL PLANTS USED FOR TRADITIONAL MATERNAL
HEALTHCARE IN KATSINA STATE, NIGERIA***

SULAIMAN SANI KANKARA

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By

SULAIMAN SANI KANKARA

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

December 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

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Chairman : Associate Professor Rusea Go, PhD
Faculty : Science

In Katsina State, Northern Nigeria, medicinal plants are widely used for the management of many medical conditions including maternal health since time immemorial. In this study, an ethnobotanical survey was conducted using semi-structured questionnaire method to obtain information on the use of medicinal plants for traditional maternal healthcare in the study area. The respondents comprised of herbalists, traditional birth attendants (TBAs), traditional medical practitioners (TMPs), housewives, farmers and others. 111 medicinal plants belonging to 101 genera, distributed across 50 families were documented. *Acacia nilotica* (L) Delile and *Guiera senegalensis* J.F Gmel had the highest Relative Frequency of Citations (RFC) and Fidelity Levels (FL). 22.52% of the cited species belong to Fabaceae family followed by Asteraceae (7.21%), Malvaceae (5.41%) and Anacardiaceae (4.51%). 25.23% of the cited species are used for general wellbeing during and after pregnancy. 68.47% of the cited species were herbs and shrubs and 84.68% of them are sourced from the wild. Leaves were the most frequently used plant's part (32.14%). 32% of the medications are prepared as decoctions and preparations are mostly (84.68%) administered orally.

A literature survey was conducted with the aid of online database, text books and unpublished theses using keywords such as "biological activities", "ethnobotanical uses", "medicinal properties", "toxicity studies" and the name of the species under review. Only species that have the combination of Relative Frequency of Citation (RFC) and Fidelity Level (FL) of at least 0.50 and 50%, respectively, were included for the literature survey. The literature survey revealed that with the exception of *Acacia nilotica*, *Euphorbia convolvuloides* and *Indigofera astragalina* DC, all the species reviewed had one or more reported biological activity substantiating its folkloric use in managing maternal health related conditions. The survey also disclosed that some of the surveyed plants are toxic. Species like *Jatropha carcus* and *Ipomoea asarifolia* were reported to be highly toxic to humans. *Acanthospermum hispidum*, *Artemisia annua*, *Citrus aurantifolia* and *Mangifera indica* were reported to have adverse effects on pregnancy in rats. *Anchomanes difformis* and *Euphorbia balsamifera* were also

reported to be highly toxic in mice and rats, respectively. No developmental toxicity of *Guiera senegalensis* was reported despite its wide use during pregnancy in the study area.

Another study was also conducted to assess the wound healing properties of *Acacia nilotica* using Sprague Dawley rats. The wound healing parameters assessed include wound contraction rate, level of proinflammatory cytokines (IL-1 β and TNF- α) and histopathological analysis of the wound area. Wounds were assessed on 7th and 14th post wounding days. The extract showed a good, dose-dependent DPPH-radical scavenging activity comparable to trolox (standard antioxidant). Topical application of *A. nilotica* cream significantly ($P < 0.05$) enhances wound contraction rate compared to the control groups in both 7th and 14th days of evaluation. The extract also significantly suppressed the expression of both IL-1 β and TNF- α in dose-dependent manner throughout the study period. The histological analysis revealed that the extract treatment enhanced tissues regeneration.

Another study was also conducted to evaluate the effects of extraction methods on the antioxidant properties of *Guiera senegalensis*. Extraction of *G. senegalensis* alone was found to be more effective than combining it with either *E. balsamifera*, *I. asarifolia* or both. Air drying appeared to be the best drying method as it produced significantly higher phenolic contents and antioxidant activities. Optimum extraction temperature was found to be 40°C, while 60 minutes appeared to be best extraction time. Although addition of natron up to 5 mg/mL was found to increase the TPC and TFC values, both antioxidant assays (DPPH, FRAP and TAC) were significantly affected adversely.

Finally, developmental toxicity of *G. senegalensis* leaves water extract on zebrafish (*Danio rerio*) embryo was also studied. Survival rate, hatching rate and teratogenic effects were used to assess the toxicity. Both survival rate and hatching rate were found to be significantly lowered by the higher doses of the extract. LC₅₀ of the extract was found to be 61.88 mg/mL. The extract also induced some structural malformations including bent tail, curved body, embryo sac edema, short body length and spinal cord curving on the hatched embryo.

This study provides the first ethnobotanical study on medicinal plants used for traditional maternal healthcare in Northern Nigeria. The results of this study if properly harnessed, hold a great potential in combating maternal mortality cases in the study area thereby achieving one of the United Nation's Millennium Developmental Goals (MDGs). Future studies directed towards conserving the reported species and standardizing traditional herbal medicine are recommended.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**KAJI SELIDIK ETNOBOTANI DAN SIFAT BIOLOGI TUMBUHAN UBATAN
YANG DIGUNAKAN DALAM PENJAGAAN KESIHATAN TRADISIONAL
IBU MENGANDUNG DI NEGERI KATSINA, NIGERIA**

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Tumbuhan ubatan digunakan secara meluas untuk banyak pengurusan kesihatan termasuk kesihatan ibu hamil sejak dahulu lagi di Negeri Katsina, Nigeria Utara. Dalam kajian ini, kajian etnobotani telah dijalankan secara kaedah soal selidik separa berstruktur untuk mendapatkan maklumat mengenai penggunaan tumbuhan ubatan untuk penjagaan kesihatan tradisional ibu mengandung di kawasan kajian. Responden terdiri daripada pakar herba, bidan kelahiran tradisional (TBA), pengamal perubatan tradisional (TMPs), suri rumah, petani dan lain-lain. Sebanyak 111 tumbuhan perubatan dari 101 genus meliputi 50 keluarga telah didokumenkan. *Acacia nilotica* (L) Delile dan *Guiera senegalensis* JF Gmel menunjukkan nilai Kekerapan Relatif Petikan (RFC) dan Tahap Fideliti (FL) yang tertinggi. Kira-kira 22.52% daripada spesies yang direkodkan adalah tergolong didalam keluarga Fabaceae diikuti oleh Asteraceae (7.21%), Malvaceae (5.41%) dan Anacardiaceae (4.51%). Sebanyak 25.23% daripada spesies yang dinamakan digunakan untuk kesejahteraan umum semasa dan selepas kehamilan. Manakala 68.47% daripada spesies yang dinamakan adalah pokok herba dan pokok renek dan 84.68% daripadanya diperolehi dari hutan. Daun adalah bahagian tumbuhan yang paling kerap digunakan (32.14%). Sebanyak 32% daripada ubat-ubatan yang disediakan adalah secara rebusan dan kebanyakan ramuan (84.68%) diambil secara oral.

Tinjauan literatur telah dijalankan dengan bantuan pangkalan data dalam talian, buku-buku teks dan tesis yang tidak diterbitkan menggunakan kata kunci seperti "Aktiviti-aktiviti biologi", "Kegunaan etnobotani", "Sifat-sifat ubatan", "Kajian ketoksikan" dan nama spesies di dalam kajian semula. Hanya spesies yang mempunyai nilai gabungan Kekerapan Relatif Petikan (RFC) dan Tahap Fideliti (FL) masing-masing sekurang-kurangnya 0.50 dan 50% masing-masing, telah dimasukkan untuk kajian literatur. Tinjauan literatur menunjukkan bahawa kecuali *Acacia nilotica*, *Euphorbia convolvuloides* dan *Indigofera astragalina* DC, semua spesies yang dikaji semula dilaporkan mempunyai satu atau lebih aktiviti biologi didalam penggunaan budaya kaum bagi pengurusan kesihatan ibu hamil. Kaji selidik juga mendedahkan bahawa terdapat beberapa tumbuhan yang dikaji adalah toksik. Spesies seperti *Jatropha carcus* dan *Ipomoea*

asarifolia dilaporkan sangat toksik kepada manusia. *Acanthospermum hispidum*, *Artemisia annua*, *Citrus aurantifolia* dan *Mangifera indica* telah dilaporkan mempunyai kesan buruk ke atas kehamilan pada tikus. *Anchomanes difformis* dan *Euphorbia balsamifera* juga dilaporkan menjadi sangat toksik masing-masing terhadap mencit dan tikus. Tiada ketoksikan terhadap perkembangan daripada *Guiera senegalensis* yang telah dilaporkan walaupun digunakan secara meluas semasa kehamilan di kawasan kajian.

Kajian lain juga telah dijalankan untuk menilai sifat-sifat penyembuhan luka *Acacia nilotica* dengan menggunakan tikus Sprague Dawley. Parameter penyembuhan luka yang dinilai termasuklah kadar penguncupan luka, tahap prokeradangan sitokin (IL-1 β dan TNF- α) dan analisis histopatologi kawasan luka. Luka telah dinilai pada hari ke 7 dan 14 selepas kecederaan. Ekstrak ini menunjukkan bahawa dos yang berkaitan dengan aktiviti DPPH-dadikal pemerangkap adalah lebih baik berbanding dengan trolox (antioksidan piawai). Aplikasi topikal krim *A. nilotica* signifikan meningkatkan kadar penguncupan luka ($P < 0.05$) berbanding dengan kumpulan kawalan pada hari ke 7 dan 14 penilaian. Ekstrak ini juga berkesan menyekat ekspresi kedua-dua IL-1 β dan TNF- α secara bergantung dos sepanjang tempoh kajian. Analisis histologi juga menunjukkan bahawa rawatan ekstrak meningkatkan pertumbuhan tisu semula.

Kajian lain juga telah dijalankan untuk menilai kesan kaedah pengekstrakan terhadap sifat-sifat antioksidan *Guiera senegalensis*. Pengekstrakan *G. senegalensis* secara berasingan didapati lebih berkesan daripada digabungkan bersama *E. balsamifera*, *I. asarifolia* atau kedua-duanya. Pengerangan udara adalah kaedah pengerangan yang terbaik kerana ia menghasilkan kandungan fenolik dan aktiviti antioksidan yang tertinggi. Suhu pengekstrakan optimum ialah pada 40°C, manakala 60 minit adalah masa pengekstrakan terbaik. Walaupun penambahan natron sehingga 5 mg/mL didapati meningkatkan nilai TPC dan TFC, namun aktiviti antioksidan (DPPH, FRAP dan TAC) menurun secara signifikan.

Akhir sekali, perkembangan ketoksikan ekstrak air daun *G. senegalensis* pada embrio zebrafish (*Danio rerio*) juga dikaji. Kadar kelangsungan hidup, kadar penetasan dan kesan teratogenik telah digunakan untuk menilai ketoksikan. Kedua-dua kadar kelangsungan hidup dan kadar penetasan dikesan signifikan diturunkan oleh dos ekstrak yang lebih tinggi. LC₅₀ ekstrak didapati pada 61.88 mg/mL. Ekstrak ini juga menyebabkan beberapa kecacatan struktur termasuk ekor bengkok, badan melengkung, pundi embrio edema, panjang badan yang pendek dan saraf tunjang melengkung pada embrio yang menetas.

Kajian ini menyediakan kajian etnobotani pertama pada tumbuhan perubatan untuk penjagaan tradisional kesihatan ibu hamil di Nigeria Utara. Keputusan kajian ini jika dimanfaatkan dengan sewajarnya, mempunyai potensi yang besar dalam memerangi kes-kes kematian ibu hamil di kawasan kajian bagi mencapai satu daripada Matlamat Pembangunan Milenium Bangsa-bangsa Bersatu (MDG). Kajian masa depan ke arah memulihara spesies yang dilaporkan dan menyeragamkan perubatan herba tradisional adalah dicadangkan.

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I certify that a Thesis Examination Committee has met on 22 December 2015 to conduct the final examination of Kankara Sulaiman Sani on his thesis entitled "Ethnobotanical Survey and Biological Properties of Medicinal Plants Used for Traditional Maternal Healthcare in Katsina State, Nigeria" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

μg	microgram
AAE	Ascorbic Acid Equivalence
ABTS	2,2'-azino-bis-6-ethylbenzthiazoline-6-sufonic acid
AChE	Acetylcholinesterase
AD	After Death
AI	Artherogenic Index
AIDS	Acquired Immuno Deficiency Syndrome
ASNAPP	Agribusiness in Sustainable African Plant Products
ATM	African Traditional Medicine
BC	Before Christ
CAT	Catalase
COX-2	Cyclooxygenase 2
CUPRAC	Cupric Ion Reducing Antioxidant Capacity
DMPD	N,N-dimethyl-p-phenylenediamine dihydrochloride
DNA	Deoxyribonucleic Acid
DPPH	2,2'-diphenyl-1-picryl hydrazyl
DRC	Democratic Republic of Congo
DW	Dry Weight
ENT	Ear Nose and Throat
EWPs	Edible Wild Plants
FL	Fidelity Level
FPV	Fowl Pox Virus
FRAP	Ferric Reducing Antioxidant Power
FSH	Follicle Stimulating Hormone
FTC	Ferric Thiocyanate
GAE	Gallic Acid Equivalence
GGT	γ -Glutamyl Transpeptide
GPx	Glutathione Peroxidase

GR	Glutathione Reductase
GST	Glutathione-s-transferase
HaCaT	Human Keratinocyte Cell Line
HAD	High Antioxidant Diet
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HORAC	Hydroxyl Radical Averting Capacity
HPLC	High Performance Liquid Chromatography
HSPs	Heat Shock Proteins
IACUC	Institutional Animal Care and Use Committee
ICF	Informant Consensus Factor
IDA	Iron Deficiency Anemia
IK	Indigenous Knowledge
IL-1 β	Interleukin 1 Beta
IL-6	Interleukin 6
IPM	Integrated Pest Management
kg	kilogram
LBW	Low Birth Weight
LDL	Low Density Lipoprotein
LGA	Local Government Area
LH	Luteinizing Hormone
m	meter
MCHC	Mean Corpuscular Hemoglobin Concentration
MDA	Malandialdehyde
MDGs	Millennium Developmental Goals
mg	milligram
mL	millilitre
mM	micromole
mm	millimeter
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

NDV	Newcastle Disease Virus
nm	nanometer
NNMDA	Nigeria Natural Medicine Development Agency
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NPC	National Population Commission
NTFPs	Non-timber Forest Plants
°C	Degree Celsius
PAI-1	Plasminogen Activator Inhibitor 1
PAI-2	Plasminogen Activator Inhibitor 2
PCV	Packed Cell Volume
PDD	Protein Deficient Diet
pg	pictogram
QE	Quarcetin Equivalence
RBC	Red Blood Cells
RFC	Relative Frequency of Citation
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
SOD	Sulforoxide Dismutase
SSDZ	Silver sulfadiazine
TAC	Total Antioxidant Capacity
TAS	Total Antioxidant Status
TBA	Thibarbutaric Acid
TBA _s	Traditional Birth Attendants
TCM	Traditional Chinese Medicine
TEAC	Trolox Equivalent Antioxidant Capacity
TFC	Total Flavonoid Content
Th-1	T-helper 1 Cells
TH _s	Traditional Healers

TLV	Traditional Leafy Vegetables
TM	Traditional Medicine
TNF- α	Tumor Necrosis Factor Alpha
TPC	Total Phenolic Content
TRAP	Total Radical Trapping Antioxidant Parameters
UK	United Kingdom
US	United States
USAID	United State Agency for International Development
WBC	White Blood cells
WHO	World Health Organization

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

Nigeria, one of the world's leading producers of oil, is Africa's most populous country and the majority of the population dwell in rural areas. Due to some reasons beyond the scope of this study, majority of the population lives below the poverty line of US\$ 1 per day (Japan International Cooperation Agency, 2011). Healthcare services in Nigeria are inadequate and unevenly distributed, and only a small number of healthcare facilities provide antenatal care, delivery and postnatal services.

World Health Organization (WHO) defined maternal death as "death of a woman while pregnant or within forty two days of termination of pregnancy, from any cause related to or aggravated by the pregnancy, irrespective of the duration and site of the pregnancy or its management but not from accidental or incidental causes." Maternal mortality is attributed to many causes such as postpartum hemorrhage (24%); indirect causes such as anemia, malaria, and heart disease (20%); infection (15%); unsafe abortion (13%); eclampsia (12%); obstructed labor (8%); and ectopic pregnancy, embolism, and anesthesia complications (8%). WHO (2014) informed that everyday about 800 women die from pregnancy or childbirth-related circumstances globally. Considering this "unacceptably" high maternal mortality figure, improving maternal health is among the eight Millennium Developmental Goals (MDGs) adopted by the international community in 2000.

Majority of maternal deaths (99%) occur in developing countries and half of this figure occur in Sub-Saharan Africa with a life time risk of 1 in every 16 women, while the figure in western countries is 1 in every 28,000 women (Nour, 2008). Access to quality healthcare has been identified as the major factor that contributes to the "vast and unjustifiable" disparities in maternal mortality between developed and developing countries (Prata et al., 2010). Postpartum hemorrhage, infection, unsafe abortion, hypertensive disorders of pregnancy and obstructed labor are the five major causes maternal deaths in developing countries (Khan et al., 2006).

The maternal mortality ratio in Nigeria is 630 per 100,000 live births, which places the country as the country with the tenth highest mortality ratio in the world (WHO, 2014). Nigeria and other five countries account for almost half (49%) of the overall maternal mortality figure worldwide (Hogan et al., 2010). This unprecedented figure cannot be disassociated from the fact that most Nigerian women employ the services of alternative traditional healthcare through the services of Traditional birth Attendants (TBAs) and Traditional Healers (THs), who inevitably use medicinal plants for maternal healthcare, particularly in rural areas where orthodox health facilities are either absent or extremely difficult to access.

Traditional Medicine (TM) defined by the WHO (1978) as “the sum total of knowledge or practices whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation to generation, verbally or in writing”, is the main source of healthcare for about 80% of African population in both rural areas- where healthcare facilities are lacking and urban areas- where treatments are offered in modern in modern health centres (Towns and Andel, 2014). People around the world, especially in rural areas utilize plants as source of medicine during pregnancy, birth and postpartum period to cure pregnancy related ailments such as miscarriage, morning sickness, swelling of the legs, abdominal pain, retained placenta, postpartum haemorrhage etc (Abdillahi and Van Staden, 2013).

Various studies (Singh et al.,1984; Browner, 1985; Bourdy and Walter, 1992; Varga and Veale, 1997; Ticktin and Dalle, 2005; Lamxay et al., 2011; Aitah et al., 2012; Nordeng et al., 2013; Borokini et al., 2013; Abdillahi and Van Staden, 2013) have documented many medicinal plants used to treat obstetric and gynaecological conditions, such as birth control, complications during pregnancy and child birth and problems associated with infertility. The extensive use of medicinal plants for traditional healthcare could be attributed to their affordability, accessibility and the fear of side effects associated with allopathic drugs. Recently, there is more interest on the use of medicinal plant crude extracts because of the believe that they contain diverse number of secondary metabolites with varied pharmacological features that act synergistically, resulting in combined effects as opposed to isolated single compound which in most cases result in adverse side effects (Mahomoodally, 2013).

Although medicinal plants are widely used for maternal healthcare around the world, most of these plants are located in remote areas and only known by the local people (Jorim et al., 2012). The surest way of introducing new ethnobotanicals into modern therapies is therefore, through reviewing the local flora and its ethnobotanical use. In spite of the long-time usage of medicinal plants, their medicinal values are mostly passed on orally from one generation to another, thereby creating a vast “legacy of undocumented materials” (Jantan, 1998). This pattern of transmission of knowledge makes traditional medicine to be vulnerable to disappearance because the custodians of the knowledge who are mostly the older people of the society, are passing away day by day and the younger generations are being exposed to modern culture acculturation (Weldegerima, 2009). This is further compounded by other human activities such as deforestation, bush burning, overgrazing etc, which directly or indirectly affect the medicinal plant diversity negatively. Documenting medicinal plants usage would therefore, go a long way in preserving the cultural heritage as well as creating information repository from which pharmacological studies aimed at production of new drug leads would be conducted.

Despite the increasing interest on the utilization of medicinal plants for traditional medicine, there is a great concern on their efficacy. This is attributed largely to the lack of scientific validation of the folkloric use of majority of medicinal plants employed for traditional medicine (Bent, 2008). One of the problems associated with Traditional Medicine as hinted by WHO (1978) is that it does not keep pace with scientific and

technological advancement and its methods are in most cases, kept secret. More so, traditional medicine is in most cases, based on ritual, mysticism and other unreal factors such as witchcraft, spiritual and moral principles which are very difficult to rationalize (Taylor et al., 2001). To provide society with novel, effective and safe drugs, pharmacological screening of medicinal plants to validate their historical usage is therefore, of utmost importance.

Depending on the type of plant utilised and ailment being treated, herbal remedies are prepared using several, mostly non standardized methods which include decoction, maceration, poultice, tincture compresses etc. These preparation methods are mostly based on cultural belief and long term experience. However, due to the diverse physical and chemical nature of the therapeutic materials, no single preparation condition is optimal for all plants at all times. Hence, there is a need to optimize the extraction conditions in order not to compromise the efficiency and efficacy of medicinal plants.

Based on inherited knowledge and long-term usage for the treatment of various ailments over the centuries and the belief that medicinal plants are natural and therefore considered safer than conventional synthetic pharmaceuticals. There is, however, scarce scientific evidence supporting this belief (Raskin et al., 2002). Recent scientific evidence has revealed that many plants considered to be medicinal are potentially toxic, mutagenic and carcinogenic (Fennell et al., 2004). Poisoning by medicinal plants may be attributed to misidentification, incorrect preparation or inappropriate administration and dosage. Information from health centres and emergency rooms has reported many dangerous and lethal effects from the use of herbal products (Rodriguez-Fragoso et al., 2008).

1.2 Objectives of the study

Taking all these into consideration, an attempt was made to document the medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. The specific objectives of this study are:

1. To carry out an ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria.
2. To review the pharmacological properties of some of the surveyed plants.
3. To scientifically validate the folkloric use of some of the surveyed species.
4. To assess the impact of processing methods on the medicinal properties of some of the surveyed species.
5. To evaluate the toxicity of some of the surveyed species.

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LIST OF PUBLICATIONS

- Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria: *South African Journal of Botany* 2015, 97: 165-175 (IF= 1.34; Q2; Scopus indexed, Published)
- Effect of drying method, solid-solvent ratio, extraction temperature and extraction time on phenolic antioxidants and antioxidant activity in *Guiera senegalensis*: *American Journal of Phytomedicine and Clinical Therapeutics* 2015, 2 (12): 1378-1392 (IF= 0.69; ISSN, Published)
- Phenolic antioxidants and antioxidant activities of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria: *African Journal of Traditional, Complementary and Alternative Medicine* (IF= 0.7; Q3; ISSN, Submitted)
- An overview on the biological properties and toxicity of medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria, *Journal of Ethnopharmacology* (IF= 2.99; Q1; Scopus index, Submitted)
- Wound healing activity of *Acacia nilotica* pods water extract in Sprague Dawley rat: *BMC Complementary and Alternative Medicine* (IF= 2.02; Scopus index, Submitted).



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