BIOLOGICALLY ACTIVE NATURAL PRODUCTS FROM ASIA AND AFRICA

A SELECTION OF TOPICS

Editor: **Anna Capasso**

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Biologically Active Natural Products from Asia and Africa: A Selection of Topics

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PREFACE

Both Asia and Africa have many plants that can be used for medicinal purposes: these medicinal plants are used in the treatment of many diseases and their uses and effects are of growing interest in western societies. Medicinal plants from Asia and Africa are not only used and chosen for their healing abilities, but also for a symbolic and spiritual meaning.

The importance of traditional autochthonous plant remedies plays a crucial role in the health of millions of people both in Asia and in Africa. Even today, traditional medicine represents the dominant medical system for millions of people showing a significant impact on health care practices. Therefore, traditional operators still represent a vital part of their health system. For this reason, even the pharmaceutical industries consider traditional medicine as a source for the identification of bioactive agents that can be used in the preparation of synthetic drugs.

This book will guide you to discover new natural products from Asia and Africa and the different ways to use them to treat or alleviate many of the most common diseases.

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CHAPTER 1

Biological and Physical Contaminants in Anti-Diabetic Herbal Medicines of Bangladesh

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Abstract: The present study evaluated biological and physical contamination in terms of microbes and toxic metal, respectively, in eight antidiabetic herbal medicines (ADHMs) from different markets in Dhaka City, Bangladesh. Coliform, *E. coli*, *Salmonella* spp. and *Listeria* spp. were absent in all ADHMs. However, aerobic bacterial count of all the samples of yeasts and molds in some samples fails to satisfy safe limits set by different regulatory standards. Among the nine metals [Copper (Cu), Zinc (Zn), Lead (Pb), Manganese (Mn), Chromium (Cr), Iron (Fe), Cadmium (Cd), Nickel (Ni), and Arsenic (As)] investigated, Cu, Zn, Pb, Cr, Ni content was in safe limit according to different pharmacopoeia and WHO guidelines. Among all the regulatory authorities, only the Health Sciences Authority (HSA), Singapore claims the Cd content is above the permissible limit in all the samples except ADHM-4. Chinese pharmacopoeia restricts the use of ADHM-1, ADHM-2 and ADHM-8 because of unacceptable arsenic (As) contamination. All the targeted antidiabetic herbal medicines (ADHMs) were found to retain an unacceptable level of Mn, ranging from 0.44±0.01 to 4.17±0.03 ppm. Metals contamination poses potential risks to human health and regulatory authorities not only should impose a restriction on the use of the medicines but also direct guidelines to keep the drugs safe.

Keywords: ADHMs, Human health and drug safety, Metal toxicity, Microbes.

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INTRODUCTION

Sometimes the conventional medicinal system fails to successfully treat some illnesses [1 - 3]. This inability results in a negative medical encounter, which badly affects the doctor-patient relationship [4 - 6]. As a result, being repulsive with conventional medicine and being attracted to some sort of values and beliefs, also referred to as postmodern philosophy, a fraction of people turns to the alternate medicinal system [4, 7, 8]. In Bangladesh, people are not required to pay extra money to the physician for a prescription and for traditional treatment, no practitioner suggests diagnosis for the investigation disorder related to physic. Therefore, people pay only for traditional drugs. Moreover, the high cost and side effects of most modern drugs shifted consumer's attention from conventional to herbal medicines [9]. Therefore, the uses of herbal medicines are increasing day by day throughout the world [10]. Consumer awareness has increased with the work of ad agencies who are airing undue respectability and credibility of herbal products on television and radio programs [11, 12]. These advertisements aim to attract the different age groups of people with their selective presentation. A child requires healthy growth and development. In his youth, the man requires to cope with daily stress and prevent or slow the onset of aging. While counting his last days on earth, the older one requires to rejuvenate himself. This journey with the requirements mentioned is incomplete without herbal remedies, which supply nutrition and essential ingredients at every step of life to maintain physic [12].

The availability of herbal remedies surpassed drug stores and entered food stores and supermarkets. About 80% of the world's population, living in the developing world, relies on herbal medicinal products as primary healthcare [9, 13, 14]. With this surge of growing use of herbal medicinal products, a whopping number of herbal preparations are incoming and concern related to safety is surging. A notable share of herbal medicine is used as antidiabetic herbal medicine (ADHM)-due to the number of people suffering from diabetes-related complications crossing 200 million worldwide [15, 16].

Metals are widely distributed in nature and occur freely in soil and water. When the metal has a relatively high density and is toxic at low concentration, it is inked as heavy metal. Among the heavy metals, mercury, lead, arsenic and cadmium are toxic metals and have mutagenic effects even at a very low concentration. Mercury was used to treat syphilis before the introduction of penicillin. Another heavy metal, arsenic, is used for the treatment of some forms of malignancy in the compound form [17, 18]. Therefore, the presence of toxic metals as a physical contaminant in herbal remedies is likely. Metal toxicity may lead to malfunction and malformation of organs. Lead poisoning may cause abdominal pain, vomiting, severe anemia, hemoglobulinuria with dark color stools [17, 18].

A wide spectrum of microorganisms has made their adobe in medicinal plants. A series of influences from animal and inanimate sources is behind this hosting. Bacterial endospore and fungal spores are prime microbial loads associated with herbal plants. These varieties of microbial load are transferred to herbal preparations. Intrinsic and extrinsic factors determine the microbial load of medicinal plants. Certain microbial contaminants may cause severe damage to human health. Certain fungal genera produce mycotoxin, which is a potential health hazard chemical. Ingestion of adherent fungal flora with herbal drugs is associated with human disorders. Not only the microbes but also the low molecular weight metabolites from molds are known as chemical contaminants. Improper handling during production and packaging may give access to microbial load to be into herbal drugs. When plastics, glass and other packaging materials come in contact with medicinal herbs, contamination takes place.

While some herbal medicines have proved potential, many of them remain un-assessed in terms of their safety and efficacy [19]. The absence of proper quality controls, improper labeling and inappropriate patient information are behind the compromised quality of herbal drugs [20]. Herbal drugs are introduced as foods or dietary supplements in some countries. By doing so, the quality, efficacy, and safety of these herbal medicines are not required to comply with drug safety regulations. If tested, then quality tests and production standards are less rigorous or controlled. Not only this, the practitioners who are prescribing the health products, may not be certified or licensed. This would leave the safety of the general public on the verge of decaying [21]. The unlicensed herbal remedy is the commonest route which does not have to meet specific standards of safety and quality, neither is it required to be accompanied by safety information for the consumer [20]. Bangladesh is one of the most populous countries, positioning eighth in the world (Fig. **1**). (https://www.infoplease.com/world/population-statistics/worlds-50-most-populous-countries-2016). With small territory, this huge population has made Bangladesh one of the most densely populated in the world.

Unsurprisingly, for a high population with limited wealth, herbal medicines are widely used as medication in Bangladesh. Therefore, the need to educate the physician as well as the general public with adequate information regarding the risks associated with the use of herbal medicines is in demand. With this understanding, a safety investigation of some antidiabetic herbal medicines (ADHMs) in terms of toxic metals (physical contaminants) and microbes (biological contaminants) was taken as our current study.

CHAPTER 2

Quantification and Health Safety Assessment of Some Toxic Metals in Anti-Diabetic Herbal Preparations Collected from Local Retailers Using the XRF Analytical Tool

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Abstract: In developing countries, an increase of diabetes became an alarming issue and recognized as the third leading fatal disorder among all syndromes. Bangladesh also has a large number of diabetic people in the world. In the present study, the quantification of major toxic metals and the assessment of their safety in the anti-diabetic herbal preparations had been undertaken. In our investigation, a handful of samples collected randomly from different kiosks and herbal retail shops in Dhaka city, Bangladesh, were exposed to the X-ray fluorescence (XRF) technique. It was found that the average concentration of calcium was the highest (660.82mg/50gm) and arsenic was the lowest in concentrations (<0.01mg/50gm) in all anti-diabetic herbal preparations (ADHPs). Cu, Fe and Ni concentration above the safety limits and two samples containing Zn concentration above the safety limits were recommended by WHO and FAO as 3 ppm, 20 ppm, 1.63 ppm and 50 ppm for herbal drugs, respectively. Other toxic heavy metals like As, Pb and Co were found with a respective concentration of <0.01, <0.012 and <0.22 mg/50 gm, which were all within their safe consumption limit. Patients who take the herbal drugs can suffer from dizziness, nausea and vomiting, dermatitis, irritation of the upper respiratory tract, abdominal pain, diarrhea, joints pain, shock, and even liver damage due to the overdose of iron and zinc.

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Based on the present study, it can be clarified that the percentage of heavy metal concentrations in herbal drugs in Bangladesh is at risk. Regulatory agencies should come forward and take the necessary measures to ensure the safety of finished herbal preparations.

Keywords: Anti-diabetic herbal preparations (ADHP), Heavy metal, X-ray fluorescence (XRF).

INTRODUCTION

Numerous diseases [1] are cured by herbal remedies due to the presence of active pharmacological components [2] in them. Eighty percent of people in the developing countries rely on herbal medicines as their primary healthcare [3, 4], and about 25% of the drugs prescribed worldwide are plant derived [5]. By 2030, 90% cases will be attributed to type-2 diabetes and their complications will reach 552 million people [6], which will be 7% of the world population. And by 2035, the number of patients from diabetes will constitute a 10% population of the world, which will be 592 million people [7, 8]. Therapeutic efficacy of relatively low cost in comparison with other medications and low side effects [9, 10] is gaining attention as ailment of diabetes type-2. Herbal formulations are inherently safe, as they are originated naturally. However, toxicity and adverse effects are not uncommon [11]. Contaminants such as pesticides, microbes, heavy metals, chemical toxins, and adulterants are held responsible for the toxicity of herbal remedies [12]. Both natural and anthropogenic sources, like the geochemical characteristics of soil and contaminants in the soil, water, and air, and others during growth, transport, and storage conditions, make a passage for the contaminants. The use of heavy metals [13, 14] in herbal formulations can have a synergistic effect [15] and amplify drug efficacy. Heavy metal, like Ni, plays an important role in insulin production [16], however, exerts a potent toxic effect on peripheral tissues and on the reproductive system [17]. For proper insulin functioning, the human body requires Mn [18]. Therefore, there is a possibility of intentional adulteration of ADHPs with Mn in the management of diabetes. Recently, toxicity due to trace metals has gained considerable attention considering their impact on human health [19 - 28].

While analyzing a sample in terms of heavy metal, the destruction of the sample bulk matrix takes place during sample preparation in popular analytical tools like ICP and AAS. However, XRF allows non-destructive analysis [29], ensuring reliability, precision and sensitivity of results when there exists a small range between deficiency and toxicity for the human body of metals. Quantification and health safety assessment of some toxic metals in anti-diabetic herbal preparations using the XRF analytical tool has been taken as a research objective.

METHODS

Study Area and Sample Collection

Anti-diabetic herbal preparation (ADHPs) is a finished commercial pack with different brands randomly collected from different kiosks and herbal retail shops of Dhaka City, Bangladesh (Table **1**). Medicines were collected in airtight plastic containers or glass bottles depending on their physical state, followed by the date of manufacturing, date of expiring and batch numbers tabulating.

Sample Preparation and Analysis

Oven-dried (35^0C for 5 minutes) samples were pulverized to a fine powder and pressed into a pellet of 13 mm size using a CARVER model manual pelletizer (6-8ton pressure). Samples were bombarded by the x-ray tube (25 V, 50 Micro A for 100 counts) and detected by a solid-state Si- Li detector. Through ADMCA and FP-CROSS software, the spectrum was analyzed. Results and figures were summarized using Microsoft Excel 2013.

Table 1. Anti-diabetic herbal preparations under investigation (n=22).

SL No	Code	Dosage Form	Dosage	Weight per Tablet or Capsule (MG)	SL No	Code	Dosage Form	Dosage	Weight per Tablet or Capsule (MG)
1	ADHP-01	Tablet	1-3 tab, 2-3 times	665	12	ADHP-12	Capsule	1 capsule, 2-3 times	505
2	ADHP-02	Capsule	1-2 capsule, 1-2 times	620	13	ADHP-13	Tablet	1-2 tablet, 2-3 times	500
3	ADHP-03	Capsule	2 capsule, 2 times	500	14	ADHP-14	Tablet	1-2 tablet, 2 times	560
4	ADHP-04	Capsule	1 capsule, 3 times	510	15	ADHP-15	Tablet	10 gms, 2-3 times	450
5	ADHP-05	Tablet	1 tablet, 2 times	500	16	ADHP-16	Tablet	2 tablets, 3 times	480
6	ADHP-06	Capsule	1-2 capsule, 1-2 times	500	17	ADHP-17	Tablet	1-2 tablets, 2 times	550
7	ADHP-07	Capsule	1 capsule, 2-3 times	450	18	ADHP-18	Tablet	1-2 tablets, 3 times	3000
8	ADHP-08	Tablet	1 tablet, 2 times	200	19	ADHP-19	Tablet	1-2 tablets, 3 times	690

CHAPTER 3

Caffeine Intake and the Risk of Female Primary Infertility: An Evidence-Based Case Report

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Abstract: Female primary infertility is a major global challenge known to be influenced by dietary factors, including caffeine intake. Moderate caffeine intake has been proposed to have beneficial health effects while excessive caffeine intake may represent health risks, with the reproductive system being one of them. However, studies regarding the association between high caffeine intake and reduced female infertility are still inconclusive. This evidence-based case report was investigated to know whether daily high caffeine consumption is associated with female primary infertility indicated by time to pregnancy (TTP) and spontaneous abortion (SAB).

A structured literature search for cohort, case-control and meta-analysis was performed using Pubmed and Scopus database. Selected articles were appraised using appraisal tools from CEBM for meta-analysis, and NOS assessment tool for cohort and case-control studies.

Four articles (one meta-analysis, two cohort studies, and one case-control study) were selected based on predefined selection criteria. High caffeine intake was not associated with 12 months TTP based on all studies, except for one case-control study. Whereas, based on the meta-analysis of 27 studies that provided sufficient data on SAB, it was shown that increased caffeine consumption significantly increased the risk of SAB. However, studies that assessed SAB had significant heterogeneity.

In conclusion, based on studies with the highest evidence level and appropriate NOS and CEBM scores, we found an insignificant association, if any, between high caffeine intake and primary infertility based on two indicators, which were TTP and SAB. Therefore, we recommend that women trying to achieve pregnancy do not necessarily need to restrict their caffeine intake.

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Keywords: Female primary infertility, High caffeine intake, Spontaneous abortion, Time to pregnancy.

CASE

A couple comes to a fertility clinic with a complaint of inability to conceive after 1 year of actively trying to become pregnant. The husband, 34 years old, is known to have 2 children from his late wife and tested normal for semen variables. The wife, 29 years old, is reported to have no secondary conditions affecting her reproductive system, however, it is reported that she had a spontaneous abortion a year before (gestational age= 7 weeks). During anamnesis, she admitted to be an avid coffee drinker who used to drink more than 5 cups of coffee/day since she was in high-school. She also heard from her colleague that a high level of caffeine contained in coffee could affect women fertility. They did not prefer to undergo medically assisted reproduction and would like to know whether the previously mentioned factor plays an important role in female infertility. The wife asked the doctor whether her high caffeine intake contributes to her infertility status?

INTRODUCTION

Primary infertility can be described by many specific definitions. It is defined as the inability to conceive after 12 months of routine unprotected sexual intercourse without a previous history of conceiving, while also defined as the inability to become pregnant or to carry out the pregnancy to a live birth [1, 2]. It is estimated that infertility has a prevalence of 9 to 18% worldwide, with an estimation of 48.5 million infertile couples in 2010 [1, 3]. Infertility is caused by both male and female factors. Female factors include ovarian disorders (*i.e.* Polycystic ovarian disease (PCOS)), endometriosis, hormonal imbalances, as well as suggested lifestyle factors such as smoking habit, alcohol, and caffeine consumption, and other dietary habits [4].

Female primary infertility can be assessed using laboratory investigations (*i.e.* biopsy and hormonal levels), imaging (*i.e.* hysterosalpingogram), as well as recorded data such as time to pregnancy [3, 5]. Time to pregnancy represents the final outcome of conception expressed in time (*i.e.* 6 or 12 months) rather than quantitative measures of biological processes observed in laboratory investigations such as hormone levels. Time to pregnancy has been proven as a cost-effective, and reliable variable to study infertility [5]. Furthermore, spontaneous abortion has also been used to evaluate primary infertility by various studies as an endpoint in which the female cannot carry out pregnancy [2].

Dietary factor has been suggested to cause infertility, with caffeine intake being one of the subjects. Caffeine (1,3,7-trimethylxanthine) is widely consumed and

found mainly in coffee (60-75%), but also in other beverages such as tea, soft drinks, and energy drinks. Moderate caffeine intake has been proposed to have beneficial health effects while excessive caffeine intake (>300-600 mg/day or 4-7 cups/day) may represent health risks, with the reproductive system being one of them [6].

Various studies have shown conflicting results of caffeine impact on various reproductive variables. A study suggests that high caffeine intake affects the free estradiol (E2) hormone, which is important for ovulation. However, the results were conflicting and inconclusive as non-significantly lower and higher free E2 levels were shown depending on race. It is suggested that caffeine affects estrogen metabolism through aromatase inhibition [7]. Furthermore, an animal study suggested that caffeine disrupts oocyte maturation by inhibiting cAMP phosphodiesterase, thus increasing the intracellular cAMP level [8]. These variables, in turn, could cause the inability to conceive, expressed by time to pregnancy. Furthermore, caffeine molecules are small enough to cross the placental barrier, and are suggested to affect endogenous hormone levels such as estradiol and progesterone in the luteal phase, while also increasing sex hormone binding globulin (SHBG) the end product of which can disrupt pregnancy and increase the possibility of spontaneous abortion [9]. Therefore, our aim is to investigate whether daily high caffeine consumption might be associated with primary infertility indicated by time to pregnancy and spontaneous abortion.

METHODS

Literature searching was done based on PubMed and Scopus, using terminologies and filters indexed in Table **1**. Duplicate studies were identified and subsequently removed; the results attained were then screened by titles and abstracts by applying both our inclusion and exclusion criteria and were subsequently checked for their full-text availability. Inclusion criteria were human studies on a female of reproductive age, with selected study designs (systematic review/meta-analysis, cohort, and case-control studies), while exclusion criteria were studies with medically assisted reproduction. Complete readings of those articles were then commenced and suitable articles were chosen accordingly. The searching results are shown in Fig. (**1**). Two independent reviewers assessed the quality of studies by employing the CEBM assessment tool for meta-analysis study, and NOS assessment tool for both cohort and case-control studies.

RESULTS

This evidence-based case report aimed to determine the association between daily high caffeine intake with primary infertility expressed with time to pregnancy (TTP) and spontaneous abortion (SAB) as the primary endpoints. From the

CHAPTER 4

An Overview of *Urena sinuata*: Phytochemistry and Pharmacological Activities

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Abstract: Urena genus consists of two species named *Urena lobata* L. and *Urena sinuata* L. These plants have various pharmacological properties, including antioxidant, anti-diarrheal, anti-parasitic, anti-inflammatory and analgesic activities and a variety of phytochemicals. *U. sinuata* is a medicinal herb, which is frequently used by the traditional practitioners in Bangladesh, India and many other countries of the world for the treatment of various diseases. The plant roots are anti-rheumatic, anti-pyretic, emollient, refrigerant, maturant, and act as a cooling agent. In this study, we summarize a detailed overview of the *U. sinuata* based on the most recent available literature (till Jun 2020). Findings suggest that *U. sinuata* possesses many important phytochemical and pharmacological activities. According to scientific reports, *U. sinuata* possesses carbohydrates and gums, reducing sugars, alkaloids, steroids, glycosides and flavonoids. Pharmacological investigations suggest that the plant has antioxidant, anti-diarrheal, anti-inflammatory, anti-pyretic, anxiolytic, analgesic, sedative, thrombolytic, insecticidal and repellent activities. In conclusion, *U. sinuata* may be one of the best sources of plant-based drugs.

Keywords: Pharmacological activities, Phytochemicals, *Urena sinuata*.

INTRODUCTION

Urena sinuata L. (Family: Malvaceae) (Fig. **1**) is also known as a sub-species of *U. lobata* [1, 2]. In Venezuelan folk medicine, Urena plant species have been utilized for their pharmacological properties, which include- anti-bacterial [3], anti-diarrheal [4], anti-parasitic [5], anti-inflammatory and analgesic [6] activities. It has been reported that a variety of compounds are isolated from this species,

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including steroids (*i.e.*, *β*-sitosterol) [7], xanthones [8], flavonoids (*e.g.*, luteolin, hypolaetin, quercetin, gossypetin, kaempferol, apigenin and chrysoeriol) and fatty acids [2].

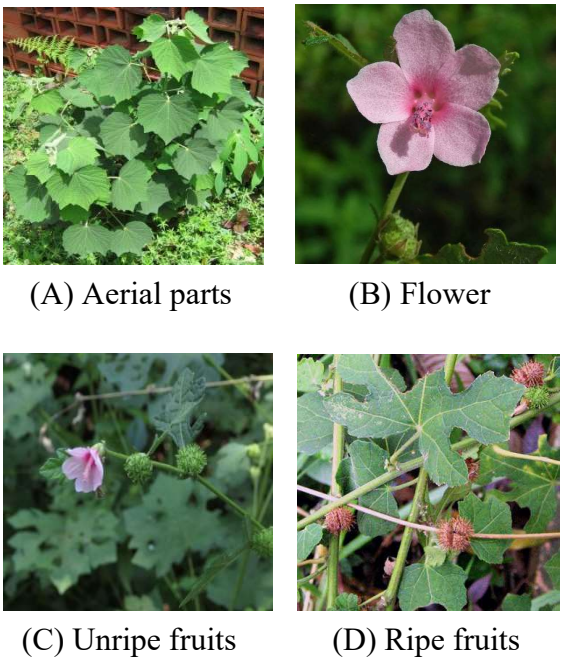
U. sinuata is a medicinal herb locally known as 'Kunjia' in Bangladesh and has a good reputation in Bangladesh, India and many other countries of the world [9]. *U. sinuata*, also known as *U. lobata* or *U. morifolia*, is a wild shrubby plant widely grown throughout the world in tropical and subtropical areas with many important folk medicinal usages [10, 11].

The plant roots are anti-rheumatic, anti-pyretic, sweet, and slightly cooling [12]. In Brazil, its stems are used in severe windy colic [13] alongside the root decoction that is used in dysentery, rheumatic pains, and tonsillitis [11] and the roots, in India, are used as an external application for lumbago and in the Pacific, Trinidad and Tobago, China and India, for reproductive purposes for both genders [14]. The roots are also considered as emollient, refrigerant and maturant in the Philippines [15, 16] and in dry and inveterate chronic coughs, the plant flowers are used as an expectorant [11]. The infusion of the flowers is used for gargles and throat bronchitis [17]. The leaves of the plant are prescribed in inflammation of the intestines and bladder. The whole plant is considered not only medicinal, but also an economic plant for various purposes in Madagascar, Nigeria and Western Sudan, Chad, Central African Republic, Zaire and Gabon for producing fiber (Aramina fiber) [15, 16].

This paper offers an up to date summary of the phytochemical and pharmacological properties of the *U. sinuata* on the basis of the database (*e.g.*, PubMed, Science Direct and Google Scholar) reports till Jun 2020.

RESEARCH METHODOLOGY

The literature on *U. sinuata* botanical description, secondary metabolites, biological properties were collected, analyzed and summarized in this review. Scientific search engines such as PubMed, ScienceDirect, SpringerLink, Web of Science, Scopus, Wiley Online, Scifnder, and Google Scholar, and various patient offices (*e.g.*, WIPO, CIPO, USPTO) were used to collect all published articles about this species. The common keyword '*Urena sinuata*', alone or paired with the 'chemical compounds', and 'pharmacological activities'. No language restrictions were imposed. The identification and manipulation of the collected data were based on their titles, abstracts and contents. Reference lists of the retrieved papers were also examined to identify further relevant papers. Chemical structures were drawn by using the Chemsketch version 12.01 software.



(A) Aerial parts (B) Flower

(C) Unripe fruits (D) Ripe fruits

Fig. (1). Images of different parts of *Urena sinuata* L.

FINDINGS

Phytochemicals of *U. Sinuata*

The plant contains carbohydrates and gums, reducing sugars, alkaloids, steroids, glycosides and flavonoids [9, 18]. Sosa and Rosquete [6] isolated and identified three quercetagetin glucosides from the leaves of the *U. sinuata*. In this study, quercetagetin-6, 7-*O*-dimethylether-3-β-D-glucopyranoside (**I**), quercetagetin-6, 7-*O*-dimethylether-4'-β-D glucopyranoside (**II**), and quercetagetin-6, 7-*O* dimethylether-3'-β-D-gluco-pyranoside (**III**) (Fig. **2**) were the major flavonoids. In another study, Sosa *et al*. [2], also report that the plant contains 6,7-di-*O*-methyl-quercetagetin- 3-*O*-*β*-D-glucopyranoside dihydrate (**I**).

Pharmacological Activities of *U. Sinuata*

Antioxidant Activity

In the cell, an antioxidant can temper the negative influence of free radicals and associated reactions [19]. Block [20] reports that antioxidants have potential

CHAPTER 5

Anti-CHIKV Activities of Diterpenes and Their Derivatives

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Abstract:

Background: Chikungunya (CHIKV) is a mosquito-borne viral disease first described during an outbreak in southern Tanzania in 1952. It is an RNA virus, belonging to the alphavirus genus of the family Togaviridae. To date, CHIKV has been identified in over 60 countries in Asia, Africa, Europe and the Americas. Diterpenes consist of two terpene units, often with the molecular formula $C_{20}H_{32,}$ and have four isoprene subunits, often known for their diverse biological effects, including anti-bacterial, anti-fungal, and anti-viral effects. Scientific reports over the past few decades, suggest that diterpenes and their derivatives can be one of the potential sources of therapeutic tools for the management of infectious diseases.

Aim: This review covers an up-to-date (2011 to July 2019) information regarding the anti-CHIKV effects of diterpenes and their derivatives on the basis of scientific evidence observed in databases.

Materials and Methods: A search was done in databases: PubMed, ScienceDirect, and Google Scholar by using relevant keywords.

Results: Findings report 121 diterpenes and their derivatives acting against CHIKV; among them, 54 were found to inhibit strongly with the EC_{50} <10 μM; while 18, 10, 10, and the rest are with 10 to <20 μM, 20 to <50 μM, 50 to <100 μM, and >100 μM, respectively.

Conclusion: More researches are necessary to investigate their possible mechanism of action behind the anti-CHIKV effect. Of note, diterpenes may be one of the important sources of anti-CHIKV drugs.

Keywords: Anti-viral drugs, Cell-based study, Chikungunya virus, Diterpenes, Diterpenoids, Terpenes.

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INTRODUCTION

Chikungunya virus (CHIKV), an arthropod-borne virus, causes an infectious disease characterized by fever, arthralgia and, sometimes, a maculopapular rash [1]. Unfortunately, we have no approved vaccine or antiviral treatment for CHIKV. To date, a number of compounds have been reported to act against CHIKV replication, but none have developed [2]. Therefore, the development of potent anti-CHIKV drugs is urgently needed.

Natural products from various origins are known to produce a vast array of terpenes. Among them, diterpenes containing various types of carbon skeletons (*e.g.*, jatrophane, lathyrane, myrsinane, ingenane, tigliane, daphnane, *etc*.) and their derivatives are of considerable interest due to their therapeutically relevant biological properties [3].

Diterpenes / diterpenoids are potent antioxidants; thus, these are generally protective in nature [3]. Strong antioxidants are also evident to act as pro-oxidants [4]. Therefore, these kinds of agents can be used as multi-edged like therapeutic tools [5]. On the other hand, the development of liposomal/nano-emulsion preparations manifests ease of administration of these kinds of drugs to the patients [6].

It is noteworthy that the CHIKV reached epidemic levels; therefore, the quest for novel and selective anti-CHIKV agents has been spotlighted today. This review focuses on the scientific data-based anti-CHIKV activity of diterpenes / diterpenoids and their derivatives.

SEARCH STRATEGY

An up-to-date search was made in the PubMed and ScienceDirect databases to access published articles up to July 2019. The relevant terms ‘diterpenes’ or ‘diterpenoids’ or ‘derivatives of diterpenes’ were paired with ‘Chikungunya virus’. No language restrictions were imposed. The search yielded 68 references. Then the list was reduced to 19 references, which were further scrutinized by reading each abstract. Attention was then concentrated on 13 papers.

FINDINGS

The articles selected for this purpose is 13. An excellent review done by Remy and Litaudon (2019) listing 110 macrocyclic diterpenoids (Table **1**) acting against CHIKV, has been included in this review, along with the 12 new papers found in the databases.

Prostratin isolated from *Trigonostemon howii* was found to act against anti-CHIKV activity (EC_{50} = 2.6 μM) [7]. *Euphorbia amygdaloides* sp. derived diterpenoids were also exhibited strong anti-CHIKV activities (EC_{50}: 0.76 ± 0.14 μM) in Vero cell-based study [8].

Norcembranoids isolated from the *Sinularia kavarattiensis* at 50 and 100 μM produced moderate anti-viral activities against CHIKV [9], while 4 diterpenoids isolated from *S. lineata* showed potent to moderate anti-CHIKV activities (EC_{50}: 1.2 ± 0.2 to 11.0 ± 0.7 μM) [10]. In another study, 4′-acetoxytonantzitlolone isolated from the stem bark of *S. lineata* sp. *lineata* was also found to act against CHIKV (EC_{50}: 7 μM) [11].

Andrographolide, a bitter diterpene lactone, derived from *Andrographis paniculata* is known for its diverse biological effects, including antioxidant, anti-cancer, and anti-metabolic syndrome [12 - 14]. In a study, andrographolide (1-100 μM) was seen to act against CHKIV in HepG2 cell-based study; the EC_{50} calculated of it was 77 μM [15]. Ten jatrophane ester of *Euphorbia* extracts were seen to act against CHIKV [16], while jatrophane ester and terracinolide J isolated from the latex of *E. dendroides* exhibited anti-CHIKV activities with EC_{50} values of 5.5 ± 1.7 and 15.0 ± 3.8 μM, respectively [17]. However, 4-deoxyphorbol ester isolated from the *E. semiperfoliata* whole plant exhibited strong anti-CHIKV activity (EC_{50} = 0.45 μM) [18]. A non-oxidized lathyrane-type diterpenoid isolated from the ethyl acetate extract of the trunk bark of *Sandwithia guyanensis* showed a moderate anti-viral activity (EC_{50} = 14 μM) against CHIKV [19]. For chemical structures of some anti-CHIKV diterpenes, please see the review article done by Remy & Litaudon [20].

Diterpenes/diterpenoids and their derivatives (along with EC_{50}) acting against CHIKV have been shown in Table **1**. For more information, please see the review article done by Remy & Litaudon [20].

Table 1. Diterpenes/diterpenoids and their derivatives acting against Chikungunya virus.

Diterpenes/Derivatives	Conc./Dose (Route)-Test System	EC_{50}/Effects	References
Prostratin isolated from *Trigonostemon howii*	Chikungunya virus (cell-based assay)	2.6 μM	[7]
Norcembranoids isolated from the *Sinularia kavarattiensis*	50 and 100 μM against Chikungunya virus (cell-based assay)	Moderate anti-CHIKV activities	[9]
4 diterpenoids isolated from *Stillingia lineata*	Chikungunya virus (cell-based assay)	1.2 ± 0.2 to 11.0 ± 0.7 μM	[10]

CHAPTER 6

Design Development and Evaluation of Anti-Inflammatory Nanogel For The Treatment of Psoriasis

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Abstract: Psoriasis is an anti-inflammatory condition associated with painful, itchy skin and typical skin lesions. Usually, psoriasis is characterized by the appearance of thick, red, scaly patches on the skin and build-up of dead skin cells leading to painful inflammation in the joints. Due to the lack of possible cure and the disadvantages of allopathic medicines, there is a need to develop new formulations from natural products having antipsoriatic activity. *Argemone Mexicana* Linn. acts as an anti-inflammatory drug for the treatment of psoriasis with no side effects as compared to synthetic drugs. Considering the anti-inflammatory activity, the attempt was made to develop a new herbal formulation for anti-inflammatory study. The developed formulations were subjected to physicochemical evaluation.

Keywords: *Argemone Mexicana* Linn, Inflammation, Nanogel, Oedema, Psoriasis.

INTRODUCTION

Argemone mexicana L, known as pila datura, belonging to family Papaveraceae, is a common weed widely distributed in many tropical and sub-tropical countries [1]. It is a herb commonly known as prickly poppy or Mexican poppy and is found everywhere in India [2]. Mexicana linn. is known as kaju and Ahon ekun. A. mexicana is considered to be one of the important medicinal plants from India. Its juice is yellow in colour which exudes when the plant is broken or injured; it is used in India as a traditional medicine for the treatment of different ailments like dropsy, jaundice and a number of cutaneous infections. Different parts like root, seed, leaves, flowers of the plant are used in the treatment of chronic skin diseases and used as an emetic, expectorant, demulcent and diuretic, *etc*. The seeds oil is used as a remedy for the treatment of a number of intestinal infections [3, 4].

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Its leaves and seeds are also responsible for maintaining normal blood circulation as well as cholesterol levels in the human body [5]. The infusion made from this plant is used in hypertension [6].

MATERIALS AND METHODS

Collection of Plant Material

The plant materials for the study included leaves and stems of *A. Mexicana.* The plant materials were collected from Latur Dist. Maharashtra in Oct-Nov and the plant materials were taxonomically authenticated by Dr. Mullani, School of life science, SRTM University Nanded, Maharashtra.

Extraction of Plant Material

Fresh plant materials (leaves and stems) of *argemone mexicana* linn were collected from the local region of the Latur district. Collected plant materials were washed under running tap water in order to remove adhering dust and other earthy matter, dried under shade and the coarse powder was prepared in a mechanical grinder. The coarse powder was extracted successively with each of the solvents with varying polarity, namely water, N-butanol and methanol, by maceration for 48 hours. The crude extract was filtered using the Whatmann filter paper and solvents were evaporated to dryness by using a water bath. Finally, dried extract was stored in a refrigerator and used for further study [7, 8].

FORMULATIONS OF NANOGEL

Preparation of Drug-Loaded Nanodispersions

Nano dispersion of the drug was prepared by the modified emulsification-diffusion method. In this method, 100 mg of the drug (extract) was weighed and dissolved in 10 ml of 10% DMSO containing tween. This was the organic phase containing drug-polymer mixture. This phase was added slowly into a 30 ml aqueous phase containing sodium alginate with constant stirring at 1000-2000 rpm using a magnetic stirrer. The addition of organic phase directly into the aqueous solution was done carefully with the help of a syringe with a needle. At this stage, the solution was stirred for at least 6 min at a continuous speed. Then distilled water was added slowly to the solution with stirring for 1 hour for the diffusion of an organic layer in the continuous phase, leading to the formation of nanodispersion [9].

Preparation of Nanogel

Gels of the nanodispersion were prepared by dispersing a gelling agent (carbopol 940) in the nanodispersion of the drug) by using a high-speed stirrer. The pH was adjusted to 7.0 by using a surfactant to form the gel, and *argemone mexicana* linn leaves and seed extract with gels were stored at room temperature [10].

Formulation Batches of Nanogel

Table 1. Formulation Composition.

Ingredients	Batches			
	F1	F2	F3	F4
Drug extract	1% (aqueous)	1% (aqueous)	1% (methanolic)	1% (methanolic)
Sodium alginate	1%	2%	2%	1%
Tween	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Carbopol 940	2%	1%	1%	2%
BHT	0.2%	0.2%	0.2%	0.2%
Propyl paraben	0.4%	0.4%	0.4%	0.4%
Distilled water	50 ml	50 ml	50 ml	50 ml

EVALUATION OF DEVELOPED FORMULATION

Appearance

All developed nanogel formulations were evaluated for homogeneity, presence of any foreign particles, and colour by visual inspection after the gels have been filled in the final container for storage.

Determination of pH

The pH of all the developed nanogels was determined using a digital pH meter. The readings were taken for an average of 3 times. The results are shown in Table **1**.

Determination of Homogeneity

All developed nanogels were tested for homogeneity; the uniformity was evaluated by visual inspection after the gels have been filled in the final container. They were tested for their appearance and presence of any foreign particles and

CHAPTER 7

Antilithiatic Properties of Moroccan Medicinal Plants and Mechanism Insights of their Phytochemicals

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Abstract: The transition from raw herbs to synthetic pharmaceuticals bioactive compounds has undergone evolution and herbal medicine has become an important source of raw materials to treat different illnesses. Indeed, the alternative treatment using herbal medicine has come into demand in recent years and has renewed interest in the plants that are effective, safe, and culturally acceptable. In Morocco, several medicinal plants are used traditionally to treat kidney stones and *in vitro* and *in vivo* experimental studies have proved their antilithiasic activity. This review aims to list all *in vitro* and *in vivo* antilithiasic medicinal plants used by the Moroccan population and to present bioactive compounds responsible for this activity. Further, we determined some molecular targets by these bioactive compounds.

Keywords: Crystallization, Kidney stones, Phyto-molecules, Urinary.

INTRODUCTION

Herbal medicine comes from the Greek word “phyto” meaning plant and “therapeuein” meaning cure [1]. Since ancient times, a series of failures and successes have been encountered in using medicinal plants for healing, pain relief, cure headaches, and heal wounds [2]. From generation to generation, the humans have passed on their knowledge and experience whose main objective was based on the idea to overcome suffering and improve their health [3].

Parts of plants used in herbal medicine produce a large number of active substances. The diversity of these substances and the fact that they are not found in all plants show that they do not involve general metabolism. So, they are known as secondary metabolites [4]. These latter comprise generally two types of

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compounds. Phenolic compounds take part in plant-plant interactions (allelopathic inhibition of germination and growth) [5] and the nitrogen compounds include alkaloids and glycosides. The latter release out hydrocyanic acid when the plants are damaged. They are synthesized from amino acids. These metabolites often play a defensive role in the plant which they manufacture [5].

A variety of structurally secondary metabolites are produced as a means for plants to defend themselves against herbivores, bacteria, fungi, and viruses. The secondary metabolites represent an exciting library of bioactive compounds filtered by natural selection, which have been used by humans to treat infections and health disorders, or as spices and perfumes [4]. Many medicinal plants have been used around the world to fight against several illnesses such as microbial diseases, metabolic disorders, and other human complications [6-9]. Numerous empirical studies using several ethnomedicinal approaches have demonstrated the efficacy of medicinal plants as a remedy against kidney illness [10-13]. Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds present in the plant extracts to treat a specific disease [14].

In this review, the antilithiasic properties of Moroccan medicinal plants have been reported. The mechanism insights of some phytochemicals against lithiasis were discussed in this review.

METHODS

The current review was done on the research of the scientific data published regarding experimental medicinal plants use against urolithiasis. The researches were obtained using various databases, including PubMed, Springer, Science Direct, Scopus, Google Scholar, Hindawi, and Taylor & Francis.

RESULTS AND DISCUSSION

Ethnobotany of Antilithiasic Medicinal Plants Used in Morocco

Ethnobotany is a discipline that reflects the relationship between human and their habitat for the purpose to search solutions against illnesses. Morocco presents a key reservoir of medicinal plants for traditional use to treat several pathologies, including urinary lithiasis. Numerous studies identifying antilithiasic plants have been reported based on ethnobotanical approaches [15 - 24].

Various traditional uses of medicinal plants have been reported in different areas of Morocco. In the Tan-Tan region, located in sub-Mediterranean bioclimatic Sahara with a temperate winter climate, Ghourri *et al.* identified 50 species with a predominant *Apiaceae* family: *Ammi visnaga*, *Ammodaucus leucotrichus*, *Apium graveolens*, *Daucus carotta*, *Eryngium triquetrum*, *Foeniculum vulgare*, and *Petroselinum sativum*. Among the use of medicinal species, the leaves and seeds have been widely used as a decoction to treat nephrolithiasis [15]. Similar plants have been reported by El Hafiane *et al*. in an ethnobotanical study conducted in the South of Morocco (Agadir). This study showed the use of *Ammi visnaga, Crocus sativus, Cynodon dactylon,* and *Petroselinum sativum* to treat kidney stones [20].

An ethnobotanical study conducted by Khouchlaa *et al.* in Western Morocco (Rabat) mentioned 35 species with a predominant *Caryophylaceae* family. The most cited plant of this family was *Herniaria hirsuta*. The Arabic vernacular name of this plant was "Harasset lahjar", which means "stones dissolution" [18]. Some medicinal plants reported in this study have not been mentioned in the study conducted in the Sahara region, such as *Aloe vera, Anthemis nobilis, Taraxacum officinalis, Caralluma europaea, Saccharum officinarum,* and *Prunus cerasus*. From this perspective, it is important to extend other ethnopharmacological studies in various Moroccan regions in order to identify all medicinal plants used for the treatment of lithiasis and safeguard the knowledge of the Moroccan population.

Other Moroccan ethnobotanical studies reported the use of medicinal plants against different diseases such as diabetes, cancer, allergy, respiratory affection, and urolithiasis. From these researches, we observed a few medicinal plants reported against urolithiasis. *Allium sativum* and *Origanum vulgare* have been reported in the north-central region of Morocco (Fès) [16]. In Settat, four plants used in the treatment of kidney stones have been cited: *Artemisia absinthium, Lavandula dentate, Zea mays, and Petroselinum sativum* [24]. In the North West of Morocco (Kenitra), *Ammi visnaga, Herniaria hirsuta*, Vicia faba, Lavandula dentate, and *Rosmarinus officinalis* have been reported [17 - 25]. In this context, further specific ethnobotanical studies on antilithiasis plants should be conducted in other Moroccan regions to identify all medicinal plants used traditionally to treat lithiasis. These perspectives can open a way for their pharmacological studies and the identification of bioactive molecules that could treat or/and prevent kidney stones.

CHAPTER 8

Ethnobotanic, Phytochemical, and Biological Activities of *Aristolochia longa* L.: A Review

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Abstract: *Aristolochia longa* is a medicinal plant used in traditional Mediterranean pharmacopeia to treat different diseases. It shows significant anti-inflammatory, antidiabetic, antioxidant, antibacterial, and antitumoral pharmacological effects. The extracts of this plant are rich in bioactive molecules belonging to different chemical families such as limonene, aristolochic acid, *β*-caryophyllene, and deenax. However, excessive use of this plant causes severe toxicity to the user. The aim of the present review is to give particular emphasis on the most recent findings on biological effects of the major groups of *Aristolochia longa* components, their therapeutic use, and the active ingredient responsible for the toxicity of this plant, which constitutes a public health problem observed with it's wide use in cancer patients.

Keywords: *Aristolochia longa*, Anticancer, Antibacterial, Anti-inflammatory, Phytochemical, Toxicity.

INTRODUCTION

Aristolochiaceae is a family for *Aristolochia,* which includes nearly 500 species for most tropical, subtropical, and Mediterranean countries [1]. This family has been reported in the forests of America, Asia, Africa, Europe, and rarely in other countries with different species depending on the country *viz*. *Aristolochia indica* in India, *Aristolochia didyma* in South Americ, Aristolochia *clematitis* in Europe, *Aristolochia heppii* Merxm in East Africa, and is known in foreign languages as Isharmul (India), Wild dutchman's pip (Americ), Saracen (France), and chivide (East Africa) [2 - 4]. *Aristolochia longa* (*A. longa*), Mediterranean species in North Africa, known as Bereztem, was recommended since antiquity against ovarian insufficiency and snake bites [5].

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It was employed in traditional medicine to treat different diseases, such as cancer, diabetes, asthma, and skin and intestinal infections, using different parts of this plant with several forms as honey, milk, and jus [6 - 8]. Recently, it was increasing used against cancer and 44% of the Moroccan population studied approved healing by this plant [9].

Herbal remedies have a huge advantage over chemical treatment. Their secondary metabolites sowed several biological activities such as anti-tumor, antibacterial, antioxidant, antifungal, anti-inflammatory, and antidiabetic [1, 6 - 8, 10 - 14]. The biological properties of this plant have been attributed to a wide range of bioactive compounds, including polyphenols, flavonoids, alkaloids, tannic acid, and fatty acids [6]. In this way, studies showed that *A. longa* possessed actives molecules, such as aristolochic acid (AA), limonene, *β*-carotene, and palmitic acid (Fig. **1**), which have proven their pharmacological effects [10, 11, 15]. However, it is necessary to carefully determine the administered dose in order to be safe.

The aim of this review was, firstly, to make a synthesis of traditional therapeutical usages of *A. longa* in disease treatment and, secondly, to give data for the most recent findings on biological activity effect. Finally, we identified the major compounds from different parts of this plant and the mechanisms responsible for their action.

CLASSIFICATION AND BOTANICAL DESCRIPTION

1. Kingdom: Plantae
2. Branch: Angiosperms
3. Class: Magnoliopsida
4. Family: *Aristolochiaceae*
5. Genus: *Aristolochia*

A. longa is a perennial plant glabrescent (20-50cm of high), with slender stems, spreading, often ramose, with triangular, oval leaves, slightly heart-shaped (3-5cm of wide), corded base, margins whole and solitary green-brown flowers (Fig. **1**). It also gives very long fruits, compared to other species of aristolochia [16].

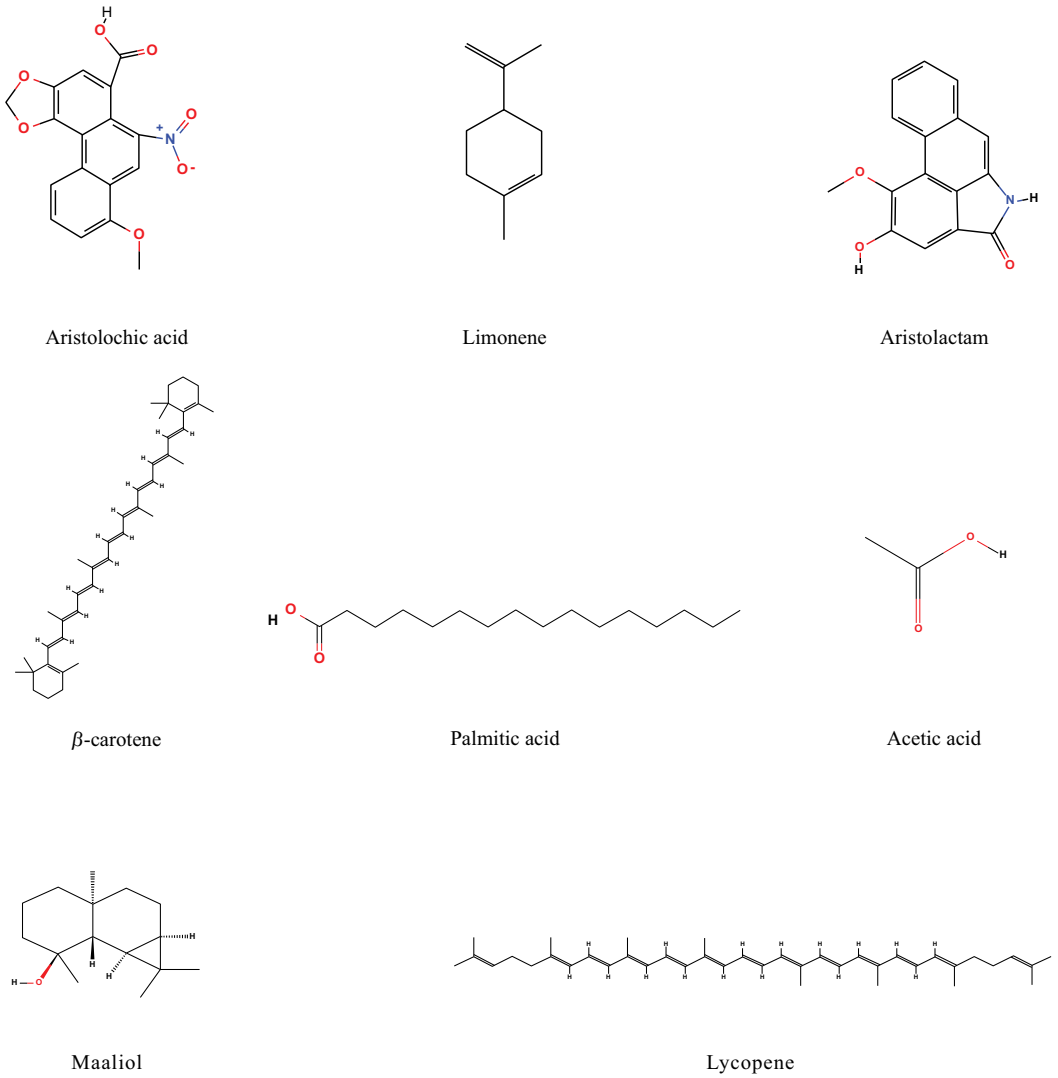

Fig. (1). Some phytochemicals identified in *A. longa*.

Ethnomedicobothany

A. longa has been used traditionally since antiquity and several ethnobotanical studies have published the use of this plant in illnesses treatment *viz*. cancer, diabetes, asthma, and digestive problems [9, 6, 17]. Table **1** summarizes the different parts and some traditional uses of *A. longa*. Depending on the area, the region, and the country, people use different parts of this plant and variable modes of preparation. The root has been used for asthma, palpitations of the aorta,

CHAPTER 9

Wound Healing Potential of Combined Extracts of Stem Bark and Leaves of *Sphenocentrum Jollyanum*: A Classical Factorial Design Model Approach

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Abstract: Stem bark, in combination with the leaves of *Sphenocentrum jollyanum,* is used for the management of wounds in the Southern part of Nigeria. The wound healing potential was determined by applying different concentrations of the prepared plant extracts, alone and in combination, to deep partial-thickness wounds on a rat model. Wound healing was measured on 15 days post-operation and compared with the controls. The percentage wound closure efficacy of the combined leaves and stem bark extracts were determined and compared statistically by 2^2 Factorial design model over 2, 8, and 15 post-operative days. Fluctuations in the wound surface pH were also measured over 15 days. All the extracts-treated wounds epithelized faster with dose-dependent wound contraction, reaching statistically significant differences ($p<0.01$) compared with untreated wounds. The stem bark extract was about 50% more potent than the leaves extract. A significantly higher wound contraction effect of combined extracts was observed when compared with the individual extract effects. Also interesting was the <10 days complete epithelization observed in combined (200 mg equivalent) leaves and stem bark-treated wounds, which is shorter than 13 post-operative days in both 100 mg stem bark extract- and cicatrin-treated groups. However, there was no statistical evidence ($*p<0.0$) of interaction between the leaves and stem bark extracts; and improved activities of the combined extracts, in comparison with the individual extracts, were purely additive. The initial alkaline wound surface pH normalized to acidic pH within 8 and 12 post-operative days in extracts- and positive control-treated wounds. *S. jollyanum* extracts possess promising wound healing property. This study validated the primary folkloric use of the plant and aside from additive effect, empirical and statistical evidences showed that there was no basis for the claimed potency of combined leaves and stem as used by the traditional healers.

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Keywords: Ethnomedicine, Factorial design, *Sphenocentrum jollyanum*, Wound contraction.

INTRODUCTION

The global burden of wound management is enormous, considering the level of illness, prolonged hospital stay, potential disability, excess costs, and sometimes death resulting from untreated wounds [1]. About 15.3 billion USD was projected to be spent annually on wound care products in the US alone by 2010 [2]; the estimate was overwhelmed at 25 billion USD was spent in 2016 alone to treat wound-related complications [3]. The situation is more disturbing in developing countries as the challenges, in terms of mortality and morbidity [4], are usually complicated by unaffordable sophisticated remedy. In Nigeria, for instance, many people still rely on medicinal plant products for the management of wounds and other ailments due to their availability, efficacy, presumed safety, and affordability [5, 6]. *Sphenocentrum jollyanum* Pierre (family Menispermaceae) is a wild tropical plant commonly distributed in Nigeria and other West African countries [7]. The plant is used traditionally in Nigeria as chewing stick, aphrodisiac and also as a remedy for cough, fever and chronic wounds [8 - 10]. Previous studies showed that various morphological parts have antipyretic, analgesic, anti-inflammatory, antioxidant, antidiabetic [11 - 15], antimalarial [16], steroidogenic, and antifatigue [17] activities. Its activity against the Polio Type-2 virus has also been documented [13]. In Nigeria, the plant is locally called Ezeogwu (king of herbs) or Orji-nkoro in Igbo, Oban Abe in Edo and Akerejupon in Yoruba languages and has been claimed to possess wound healing activity [9], which is yet to be validated. This claim has been sustained in Southern Nigeria by the African traditional and complementary medicine (ATCM) practitioners due to its ethnotherapeutic potency. These traditional healers claimed, in addition, that its wound healing property is evidenced only when both the leaves and stem bark are ground together with few drops of water and the squeezed pulp from the paste is applied to fresh wounds.

The validity or otherwise of these claims is still unknown. This study, therefore, seeks to (i) investigate separately the wound healing properties of the aqueous extracts of stem bark and leaves, and further (ii) determine the wound healing potential of these combined aqueous extracts with the objective of validating the folkloric claims of the herbal uses of *S. jollyanum* leaves and stem bark.

MATERIALS AND METHODS

Collection and Processing of Plant Materials

The plant materials (fresh stem bark and leaves) were collected in 2018 with the help of a traditional healer from a forest in Benue State Nigeria (N 7 43' 50", E 8 32' 10"); and identified and authenticated by a taxonomist at the Institute of Plant Science and Biotechnology, University of Nigeria Nsukka. A voucher specimen (UNH 302m) was prepared and stored at the herbarium of the Institute for future reference. The plant name was further confirmed at http://www.theplantlist.org on 20th December 2018.

Preparation of The Plant Extracts

The plant extracts were prepared in line with the ethno-pharmacological information obtained from ATCM practitioners and local users. A 1000 g each of the fresh plant materials was washed with distilled water and allowed to drain completely under the shade. The plant materials were pulverized and the extract was squeezed with fresh distilled water. The mixture was filtered thereafter and the filtrates were concentrated to dryness by freeze-drying. The dried extracts were preserved at 4 ºC until use. The application of the plant extracts was also done according to the traditional healers' instructions by placing directly on the fresh wounds as described subsequently.

Phytochemical Analysis of Plant Extracts

The crude extracts of *S. jollyanum* were tested for the presence or absence of major secondary metabolites such as glycosides, saponins, anthraquinones, flavonoids, terpenoids, tannins, alkaloids, and reducing the sugar by standard method [18]. Quantification of secondary metabolites in both extracts was performed by standardized methods [18 - 21].

Experimental Animals and Test Groups

Albino rats (±50 g) of either sex were used for the excision wound healing study and were divided into treatment groups of five rats each, as described below. All the experimental animals were acclimatized for 14 days, with free access to standard animal feed and water, distinctively marked and weighed prior to the study. All the animals were handled in line with the University of Nigeria Ethics Committee guidelines on the handling of laboratory animals and other related products.

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Professor Anna Capasso received her Degree in Pharmacy (1986), Master in Pharmacology (1988), Ph.D. in Pharmaceutical Sciences (1992) from the University of Naples Federico II, Italy. During her Ph.D. studies, she studied the interferences between glucocorticoids and the opioid system. From 1993 to 2002, she was the researcher in Pharmacology. From 2002 till today, she is an associate professor of Pharmacology at the University of Salerno, Italy. Professor Capasso is the founder and editor-in-chief of Pharmacologyonline. She is also an editor -in-chief of the Open Biochemistry Journal and an associate editor of the Open Neurology Journal. She is also the member of the editorial board of Basic & Clinical Pharmacology & Toxicology, Phytotherapy Research, Current Medicinal Chemistry, Current Medicinal Chemistry-Central Nervous System Agents, Letters in Drug Design & Discovery, Medicina, Biomedical Research, and many others. Prof. Capasso is also the member of the Italian Society of Pharmacology, the Italian Society of Neurosciences, and the International Narcotics Research Conference Group. Prof. Capasso's research is documented by about 500 publications (H-index: 28), including full papers, book chapters, and congressional papers. Her research topics are: neuropharmacology, neuroscience, neurology, psychiatry, and addiction.