Anti-microbial and immunomodulatory properties of indigenous plants found in Central and Southern Africa

AN. Traoré[1], MT. Sigidi[1], MM. Boukandou[1], MI. Ntlhamu, R. Musoliwa, MP. Tshisikhawe[2] and N. Potgieter[1,3*]

[1] Department of Microbiology, University of Venda; Private Bag X5050; 0950 Thohoyandou, South Africa
[2] Department of Botany, University of Venda; Private Bag X5050; 0950 Thohoyandou, South Africa
[3] Office of the Dean, School of Mathematical and Natural Sciences; University of Venda; Private Bag X5050; 0950 Thohoyandou, South Africa

Plant-derived medicinal products are currently in high demand both by traditional healers and the herbal drug industries, and their popularity is growing. In developing countries, traditional medicine is used to meet the primary health care needs where infectious diseases are endemic and modern health facilities and services are inadequate. In both Gabon and the Venda Region of South Africa, indigenous plants have been used for the treatment of several ailments like malaria, jaundice, diarrhoea and HIV/AIDS. Some medicinal plants are reported to have immunomodulatory properties; but a large number of indigenous plants such as *Cassia abbreviata, Copaifera relogiosa, Costus lucanusianus, Ziziphus mucronata* and *Lannea edulis*; have yet to be thoroughly investigated. Studies done in some countries have reported on their biological activities in order to justify their utilization in traditional medicine and few investigations have been carried out on their anti-microbial and immunomodulatory potentials. This review aimed to highlight studies done on these indigenous plants based on information compiled from scientific databases (Science Direct and PubMed) and the most recent results obtained in house.

Keywords: Anti-microbial, immunomodulation, indigenous plants, primary health, traditional medicine.

1. Introduction

1.1 Background

Medicinal plants have been used throughout the history of humans [1]. In Africa, the population uses traditional medicine for the treatment of various diseases and ailments like malaria, typhoid, ulcers, skin diseases, diabetes, reproductive problems, aches and pains for various socio-cultural and economic reasons [2,3]. In addition, it is estimated that 80% of the Asian and African populations use herbal medicine as aspect of primary health care (World Health Organization [4].

Plants are able to produce different kinds of chemical compounds for performing different biological activities and defence against insects, fungi, animals, and microbes. Nowadays, natural products of plant sources have been the centre of focus as the main source of new, safer and effective bioactive compounds with medicinal properties. Medicinal plants possess ingredients which can be used to develop drugs and synthesize them [5]. Most of the prescribed drugs used today are made from plants [2].

Indeed, several natural products and their derivatives constitute over 50% of all drugs with various chemical structures and disease preventive properties. These compounds also called phytochemicals or secondary metabolites which include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others [6]; are known to be responsible for several pharmacological activities of plants such as anti-malarial, anti-asthma, anti-cancer, cholinomimetic, vasodilatory, anti-arrhythmic, analgesic, anti-bacterial, and anti-hyperglycemic activities [7-10]. The selection of plants based on their ethno-medicinal usage increases the likelihood of finding an effective therapeutic agent as opposed to random selection of plants [11,12].

New chemotherapeutic compounds which are effective and of low cost are urgently needed [13]. In recent days herbal products represent safety compared to synthetics which are considered to be harmful to people and their surroundings [14]. Moreover, the dependence on synthetics is over and people are returning to natural remedies with hope of safety and security [14].

South Africa like the rest of Africa has a rich diversity of more than 24 000 indigenous plant species, representing approximately 10% of all higher plants on Earth [15]. The people of South Africa have a long tradition of medicinal plant use with an estimated 70% of the population harvesting one if not more from the approximated 3 000 species of plants used as traditional medicines [15-17]. The country's vast variety of indigenous floral species together with their diverse medicinal uses indicates a great potential for uncovering new bioactive chemicals [3,18,17].

Over the past few years, various research groups from different fields have investigated the ethnobotanical, chemical and health characteristics of medicinal plants in the Vhembe district from the Venda region of South Africa [19-21]. The traditional knowledge of plants and animals in relation to their use within the district ought to be documented for future generations [22]; however, it has not been well explored scientifically. The shortage of scientific evidence allows the native medicinal plants of Africa to be considered as relatively unexploited potential sources of novel anti-retroviral compounds [23].

A number of surveys have highlighted the fact that rural people heavily rely on traditional medicine for treatment of many ailments [24,20,25-27]. The use of such plants could be attributed to their attainability, affordability [28] and cultural beliefs [29]. For some villagers, the dependency on medicinal plants is due to lack of access to modern medical facilities or lack of effective services within the facilities if ever present.

It is also of great importance to understand the mechanisms of the immune system during infection to help improve its capacity to defend itself against subsequent infections. Few studies have been conducted to assess the effects of medicinal plants on the immune system of those diagnosed with various life threatening infections. A study conducted by Bessong et al [21], evaluated the biological activities of *Terminalia sericea, Bridelia micrantha* and *Combretum molle* against HIV-1 (reverse transcriptase (RT) and RnaseH). Another study was conducted to evaluate *Bridelia micrantha* activity against viral enzymes [30]. Additional efforts have also been made to investigate the immunomodulatory effects of *Combretum hereroense* and *Canthium mundianum* [20].

The current paper aimed to highlight studies done on these indigenous plants based on claims by traditional healers on their healing properties against life threatening infections and the most recent results obtained in house.

2. Methods used

2.1 Literature search on plant materials

The focus of this review was on *Cassia abbreviata*, *Copaifera relogiosa*, *Costus lucanusianus*, *Ziziphus mucronata* and *Lannea edulis* used as therapeutic plants. The search term used was "*name of plant*", on several online databases such as PubMed, Science Direct, and Google. The information found from this search was then matched with information gathered from interviews with traditional healers. Then, a compilation of each plant on their description usages and pharmacological activities such as anti-microbial, anti-cancer, anti-oxidant, and toxicity was done.

2.2 Reagents and equipment

RPMI-1640, foetal calf serum (FCS), streptomycin and L-glutamine were purchased from *Thermo Fisher* (SA). Blood was obtained from volunteer donors from the University of Venda community. Histopaque 1077 and the MTT salts were purchased from *Sigma Aldrich* (SA). All chemicals were bought from *Merck Chemicals* (SA). The culture plates and plastic ware were obtained from *AEC Amersham* (SA). The ELISA kits were purchased from *BD Biosciences* (San Jose, CA, USA). The 96-well plate reader was acquired from *Separations* (SA). Most laboratory equipment was purchased from NETZSCH (Germany) and BÜCHI Labortechnik AG (Switzerland).

2.3 Collection of plant materials

Medicinal plants were selected on the basis of their intense use for the treatment of different ailments and also a literature search was conducted to corroborate the scientific findings with traditional findings. Numerous traditional healers were interviewed to understand how they treat and "prescribe" medications for their patients.

Stem barks of *Copaifera religiosa* and stems of *Costus lucanusianus* were collected in Libreville (Gabon) after being identified by a botanist of the National Herbarium of Gabon.

Cassia abbreviata, Ziziphus mucronata and *Lannea edulis* were collected from within the geographic locations of the Vhembe District Municipality of the Limpopo Province (South Africa) with assistance from a traditional leader. These plants were identified by their vernacular names then later using taxonomic keys by an ethnobotanical specialist at the University of Venda (Limpopo province; South Africa). Voucher specimens were deposited in the herbarium of the Department of Botany (University of Venda).

The plants were dried under the sun light, grounded and then stored in a cool dark place until further use.

2.4 Plant preparation and extraction

Extraction was performed using water and methanol or Ethyl Acetate. After 24 h maceration and filtration, the aqueous filtrate was frozen and water was removed by freeze drying.

The methanolic or ethylic filtrates were evaporated by rotary evaporation at 50°C. The crude extracts obtained were then stored.

2.4 Phytochemical analysis

Both aqueous and methanol or ethyl acetate extracts were completely dissolved in their respective solvents (water and methanol or ethyl acetate). The obtained stock solutions were used for phytochemical screening as reported by Harborne [31] and Hossain et al [32] using chemical standard methods. The methods used are colorimetric assays that depend on color change of plant extracts after reaction.

2.5 Antimicrobial assays

2.5.1 Microorganisms tested

The selected strains for the current study were *Staphylococcus aureus* (ATCC 25923), *Salmonella enterica* (ATCC 51741), *Escherichia coli* (ATCC 25922), *Shigella sonnei* (ATCC 25931), *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (ATCC 13047), *Streptococcus agalactiae* (ATCC 12386) *Enterococcus faecalis* (ATCC 29212), and *Bacillus cereus* (ATCC 10876) and were obtained from ANATECH (SA). The drugs used as positive controls were Gentamicin sulphate, Vancomycin hydrochloride, Imipenem dehydrate, Amicillin, Penicillin G and Chloramphenicol.

2.5.2 The well diffusion assay

The different plant extracts were studied for anti-microbial activity against pathogenic microorganisms using agar well diffusion assay as described by Perez et al [33] with some modifications. Antimicrobial activity of extracts was tested against Gram-positive and Gram-negative strains.

The test strains were cultured by inoculation into sterile nutrient agar plate (Mueller Hinton media) and incubated overnight at 37 °C. The cultured strains were transferred into test tubes with 5 ml of sterile water and the turbidity of the inoculumn was measured using the McFarland standard of 0.5.

The test bacteria were aseptically swabbed on nutrient agar plates using sterile cotton swabs. With the help of a sterile cork borer, wells of 6 mm diameter were punched in the inoculated plate. 50 µl of extracts at concentrations of 200, 150, 100 and 50 mg/ml were tested against the strains, and then incubated overnight at 37 °C. The plates were examined for the zone of inhibition, which appeared as clear areas around the wells.

Inhibition zone diameters were recorded.

2.6 Testing for immunomodulatory effects of the plants

Whole blood from healthy volunteers (collected in EDTA coated tubes, ethics approval number SMNS/14/MBY/30/1210) was used for the isolation of peripheral Blood mononuclear cells (PBMCs) as described by Ngcobo et al [34].

PBMCs (5 × 105) in culture medium (RPMI 1640 containing FBS 10% and 100 µg/ml of streptomycin) were added to a 24 multi-well culture plates and treated with 50 and 100 µg/ml concentrations of *Cassia abbreviate, L. edulis* and *Z. mucronata* extracts for 72 h in 5% CO_2 atmosphere at 37 °C. Three control groups consisted of PBMCs in medium as baseline control, PBMCs in media containing DMSO as negative control and PBMCs in medium plus PHA as positive control were used.

After incubation, the supernatant of cell cultures were used to assessed the production of cytokines (IL-2, IL-6 and TNF-α) according to the manufacturer's instructions and the cells were used for viability using the MTT assay (as described by Mosmann [35]. All experiments were carried out with three different donors and each test was performed in triplicate.

3. Finding and Discussion

3.1 Description of the indigenous plants

This section gives the ethno-botanical and pharmacological description of *Cassia abbreviata, Copaifera relogiosa, Costus lucanusianus, Ziziphus mucronata* and *Lannea edulis* as summarized in Table 1.

3.1.1 *Copaifera religiosa* J.Leonard

Description: *Copaifera religiosa* is a species belonging to the family of Caesalpineaceae (Leguminosae) family. It is a tree with a height of 46 m. The bole is straight, cylindrical, and up to 30 m in length, with swollen base in older trees. Buttresses are absent. The trunk diameter reaches up to 200 cm [36]. It mostly occurs in dense, primary forests and more rarely in the sedimentary basin in mixed stands

Uses: According to Brink [37], traditional uses of this plant include treatments against malaria symptoms, cardiovascular diseases, stomach ache, and cough. *Copaifera religiosa* is utilized in Gabon to treat malaria, whereas the bark is used in fumigations against headache and kidney pain and it is added to a bath together with other ingredients to treat leprosy [37,38].

Table 1 Properties of the selected plants

Family	Botanical name	Plant parts	Traditional used	Pharmacological properties	Toxicity	Phytochemical composition
Caesalpiniaceae	*Copaifera religiosa* J.Leonard	Roots, Leaves, Stem bark, Fruits	Abdominal pains, dysentery, fever, malaria, hernia, wounds, impotency, snake bite, diarrhoea, aphrodisiac, arbotifacient, venereal diseases, stomach ache, skin rashes AIDS, sterility, cough, emetic, epilepsy, bilharzia, jaundice, hernia	Antibacterial, antifungal, Antimalarial, Antiviral Anthelmintic, Antioxidant, Antidiabetic, anti-inflammatory, antiHIV	Not cytotoxic on PBMCs but toxic in shrimps.	Alkaloids, saponins, phenols, flavonoids, anthraquinones, glycosides, coumarins, sterols and alcohols derivatives
Caesalpineaceae (Leguminosae)	*Cassia abbreviata* Oliv	Barks	Malaria, cardiovascular diseases, stomach ache, cough, sterility, head ache, leprosy, kidney pains	In vitro antiplasmodial	Cytotoxic	Not found in the literature
Costaceae	*Costus lucanusianus* J.Braun & K.Schum	Inflorescences, Leaves, Stems, Juice	Filariasis, tachycardia, stomach ache, cough bronchitis, eye troubles, headache, oedema, fever, urethral discharges, jaundice	Anti-inflammatory, antioxidant, cytotoxic, antihyperglycemic, hepatoprotective, renoprotective, antidiarrheal, antiabortive, antimicrobial	Hepatotoxic	Glycosides, tanins, saponins, reducing sugars, flavonols carbohydrates, myricetin
Anacardiaceae	*Lannea edulis* Engl.	Leaves, Roots	Stomachaches, Wounds, diarrhoea, eye troubles	Antioxidant, antimicrobial	Moderately toxic	dihydroalkyl-hexenones Flavonoids, tanins alkylphenols, cardonols,
Rhamnaceae	*Ziziphus mucronata* Willd	Roots, Leaves, Barks, Fruits	Emetic, cough, skin disorders, pain, diarrhoea, venereal diseases, stomach ulcers rheumatism, respiratory infections, gastrointestinal complaints, snake bites, swellings, wounds, diabetes	Mutagenic effects, antioxidant, antimicrobial, α-glucosidase and α-amylase inhibitor, antidiabetic	Not toxic	cardiac glycosides, saponins, flavonoids, proanthocyanidin, tannins

Known Research Findings: It has some interesting anti-plasmodial activity and its dichloromethane bark extract has shown considerable in-vitro cytotoxicity [38]. A phytochemical screening has demonstrated the presence of tannin [37].

3. 1. 2 *Costus lucanusianus* J.Braun & K.Schum

Description: *Costus lucanusianus* J.Braun & K.Schum is also called Spiral Ginger. The plant belongs to the family of Costaceae [39]. Spiral ginger is a perennial, rhizomatous herb, which attains up to 3 meters in height. This plant is mainly found in Africa specifically in DR Congo and Tropical western Africa – Guinea east to western Ethiopia.
Uses: The plant is mostly used for religious and ceremonial purposes [40-42]. Inflorescence infusion treats tachycardia and stomach aches [43]. A decoction of a stem or fruit is used for the treatment of cough, bronchitis and a sore throat. Leaf sap treats eye troubles, headache, oedema and fever [43,44] and can be used as nose drop. Leaf pulp can slow down insanity when it is rubbed. Stem sap is also able to cure/treat urethral discharges, jaundice, and also to prevent miscarriage [45]. In Gabon, stem sap controls filariasis when applied as eye drops [46].
Known Research Findings: Numerous studies have been done on *C. lucanusianus* based on their ethnobotanical uses. *C. lucanusianus* display many pharmacological activities such as anti-inflammatory activity, anti-nociceptive activity, anti-abortive activities, dysmenorrhea, pyrexia, anti-diarrhoeal activity [47]. *Costus lucanusianus* possesses phytochemical compounds such as tannins, saponins, reducing sugars, carbohydrates, myricetin, and flavonols [48].

3. 1.3 *Cassia abbreviata* Oliv.

Description: *Cassia abbreviata* (Caesalpiniaceae) is a shrub which grows up to 10 m in height. It has a light brown bark, rounded crown and yellowish leaves. It has compound leaves, with 5 to 12 pairs, and brown black pods which are cylindrical in shape. Flowers are yellow, sweet-scented, large, loose, becoming brown-veinted with age and fruits are long cylindrical dark brown and hanging pod [49]. It is widespread in Africa, from Somalia to South Africa and occurs mostly at low to medium altitudes (between 220 and 1520 m above sea level), in open bushveld, woodland or wooded grasslands, along rivers, on hillsides and frequently on termite mounds [50].
Uses: In South Africa and Botswana the root is ground into powder, mixed with water and used to wash dirty blood, referring to a woman who has miscarried and need to be cleansed [51]. In Tanzania, the decoction of the root is made to treat abdominal pains, dysentery, fever, malaria, hernia, wounds, syphilis, impotency and snake bite [52,53]. In Mozambique, the decoction of root bark may be taken orally to treat diarrhea [54]. It is an aphrodisiac and is an arbotifacient [17]. Decoction of stem bark is taken orally to treat stomach ache and malaria, while infusion of roots, leaves and stem bark mixed together is taken to treat stomach ache [54]. Bark and roots may also be used to treat stomach ache of a mother during pregnancy, close fontanelle of newborn babies, dysentery, blood vomits, venereal diseases, bilharzia, hernia, post-partum pains and menstrual cycle problems [55]. It may also be used to treat skin rashes associated with human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) infections [56].
Known Research Findings: Many studies have demonstrated its various pharmacological properties such as anti-malarial, anti-helmintic, anti-viral, anti-oxidant, anti-diabetic, anti-bacterial and anti-fungal activities [57]. Methanol extracts of the root exhibited high toxicity in a brine shrimp lethality test [58]. Ethanol root extracts did not show cytotoxicity on PBMCs [59]. Its phytochemical composition includes alkaloids, saponins, phenols, flavonoids, anthraquinones, glycosides, coumarins, sterols and alcohols derivatives [59].

3.1.4 *Ziziphus mucronata* Willd.

Description: *Ziziphus mucronata* is a small to medium-sized tree species with a spreading canopy. It is commonly known as the Buffalo thorn, a species in the Rhamnaceae family. It is distributed throughout the summer rainfall areas of sub-Saharan Africa, extending from South Africa northwards to Ethiopia and Arabia. It is up to 8 m tall with a rounded crown, which grows on loamy sands. The leaves are three veined from the base and often; there is one straight and one hooked spine at the leaf axil. The flowers are said to be small and yellowish forming clusters. The fruit is almost circular, about 1.5 cm in diameter and bright red when ripe. The fleshy drupes are rich in sugars and vitamins [60]. The plant is common at medium altitudes mainly in mixed woodland, in areas with medium to lower rainfall, also on termite mounds.
Uses: The Zulu nation takes the powdered leaf and bark in water as an emetic in chest troubles. They also use hot infusions of the bark for cough. In general a poultice of the leaf is applied to boils, carbuncles and other septic swellings of the skin. Decoction of the root is taken internally and a paste of the leaf is applied to tubercular glandular swellings. For Pain of any sort, a poultice of meal made with a decoction or of powdered baked root is applied. People do inhale the vapor and gargles with a decoction of the leaf and shoot for measles and scarlet fever [61]. The roots can be used to treat bloody diarrhoea or stomach ulcers. *Ziziphus* is used to treat gonorrhea [60]. The root decoction is taken internally and a paste of the leaf is applied to tubercular glandular swellings. Hot infusion of the bark is used for coughs and the powdered leaf and bark in water is used as an emetic in chest troubles.

Known Research Findings: *Ziziphus mucronata* in the presence of metabolic activation showed mutagenic effects [62]. The phytochemistry of the plant has not yet been exhaustively studied; current literature survey showed one other article indicating that the leaf extract contains low amounts of tannins [63].

3.1.5 *Lannea edulis* Engl.

Description: *Lannea edulis* is a species that belongs in the Anacardiaceae family. It is a small perennial shrub, which grows from, branched underground stems 30-300mm high from large woody root-crown, or from a creeping rhizome. It occurs on deep sand and grows up to 20cm tall. The alternate pinnate leaves have 5 to 7 pairs of shiny leaflets which are variable in shape and size. The flowers are small, about 2mm long and yellow borne in a congested false spike or panicle and can be seen in September. The fruits are about 1cm long, green but turning red when ripe. They occur from September onwards and can be seen on the plant at same time as flowers [60]. The shrub is common in most Zimbabwean areas and is found in open woodland. The fruit which is purplish-black in color when ripe has a juicy pulp and is pleasantly sour.
Uses: Traditionally the leaf infusion is used to treat stomach aches. The powdered leaves are applied on wounds for healing. A cold infusion of the roots of *Lannea edulis* is used for treating diarrhoea. Africans take a decoction of the roots particularly of its bark in frequent large doses for blackwater fever. The Lobedu use a cold infusion of the leaf as a local application to the eyelashes to loosen the dried pus in sore eyes [64].
Known Research Findings: It has been found that the dichloromethane root extracts of *Lannea edulis* do contain some important phytochemical groups like alkylphenols, cardonols and dihydroalkylhexenones [65]. Lannea has also been shown to be able to induce frameshift mutations in Salmonella [66].

3.2 Phytochemical screening

In this study, the phytochemical analysis of the methanol and aqueous extract of *Copaifera religiosa* and *Costus lucanusianus* showed the presence of different groups of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, saponins, terpenoids, steroids and phenolics (Table 2). They were present in both aqueous and methanolic extracts of *Copaifera religiosa* and *Costus lucanusianus.* The presence of glycosides and Saponins in the methanolic extract of *C. lucanusianus* correlates with the phytochemical results obtained respectively by Baba and Onanuga [41] and Owolabi et al [42].

Phenolics were only present in the methanolic extract of *Copaifera religiosa.* Alkaloids in *Copaifera religiosa* were found only in the aqueous extracts while in *Costus lucanusianus* it was the methanolic extracts which contained them. Saponins were present in both aqueous and methanolic extracts of *Copaifera religiosa.* But in *Costus lucanusianus,* saponins were present only in the aqueous extract.

Table 2 Phytochemical screening on the methanol and aqueous extracts of the bark parts of *Copaifera religiosa* and *Costus lucanusianus*

Class of compounds	***Copaifera religiosa***		***Costus lucanusianus***	
	Aqueous	Methanolic	Aqueous	Methanolic
Flavonoids	+++	+	++	+++
Steroids	+	++	+	++
Cardiac glycosides	++	+++	+	++
Phenolics	-	+++	-	-
Saponins	++	+	+	-
Terpenoids	+	+++	+	++
Alkaloids	+	-	-	+

+ = Presence of constituents (+++= High, ++= Moderate, += Low); – = Absence of constituents

Furthermore, the phytochemical analysis of the aqueous and ethylic extracts of *Cossia abbreviata* and *Lannea edulis* revealed the presence of cardiac glycosides, flavonoids, saponins, steroids and phenolics as mentioned in Table 3.

Both the ethyl acetate and water extracts yielded the same compounds except for saponins which were positively tested in the water extracts only. The tested plant extracts exhibited 100% positive presence for phenols, flavonoids, steroids, cardiac glycosides and saponins was present in all the water extracts. *L. edulis* possessed the most phenol concentration whereas *C. abbreviate* possessed more flavonoid.

The extracts of *C. abbreviatta* had higher concentrations of saponins. Both plants showed higher concentration of cardiac glycosides.

Table 3 Phytochemical screening on the Ethyl Acetate (EtAc) and aqueous extracts of *Cossia abbreviata* and *Lannea edulis*

Class of components	*Cassia abbreviata*		*Lannea edulis*	
	Aqueous	EtAc	Aqueous	EtAc
Phenolics	++	++	+++	+++
Flavonoids	+++	++	+	+
Saponins	+++	-	+	-
Steroids	+++	+++	+	+
Cardiac glycosides	+++	+++	+++	+++

+ = Presence of constituents (+++= High, ++= Moderate, += Low); – = Absence of constituents

The phytochemical constituents of the plants tested demonstrated compounds including steroids, flavonoids, cardiac glycosides, phenols and saponins. These compounds are known to display medicinal activity as well as physiological activity [67]. The presence of different phytochemicals in the extracts accounts for varying effects they exert either directly on the pathogens or by neutralizing the body's defense by-products through anti-oxidation. Researches have shown that saponins are well known to possess detergent properties and to exhibit anti-inflammatory properties [68].

Furthermore, cardiac glycosides have been reported to increase the concentration of Ca^{2+} ions by inhibiting the Na^{+}/K^{+} pump. The increase of such ions controls the contraction of the heart muscles thus has been employed in the treatment of congestive heart failure [69].

Moreover, flavonoids have been reported to be highly potent antioxidant compound that aids in the reduction of stroke incidences, cancer and heart failure [67].

3.3 Anti-microbial activity of selected plants

The antimicrobial activity of the crude extracts (methanol and aqueous) of *Copaifera religiosa* and *Costus lucanusianus* were studied at different concentrations against 9 pathogenic gram (+) and gram (-) bacteria (*E. faecalis, B. cereus, S. aureus*, *K. pneumoniae, S. sonnei, S. enterica, E. cloacae, P. aeruginosa, E. coli*).

C. lucanusianus aqueous extract did not show any inhibition against all strains, while methanol and aqueous extract of *C. religiosa* exhibited potent antimicrobial activity towards *B. cereus, S. aureus, E. faecalis*, and *P. aeruginosa*. The zones of inhibition values compared with gentamicin as positive control are presented in (Table 4).

S. aureus was found to be more susceptible towards the aqueous extract of *C. religiosa* with a maximum inhibitory zone (33.33±1.52 mm), followed by methanol extract (23.33±1.52mm). *B. cereus* was found to be more sensitive to the methanol extract with a maximum inhibitory zone (15.66±0.57mm),

E. faecalis was found to be more sensitive to the *C. religiosa* methanol extract, with a maximum inhibitory zone (10.66±0.57mm), followed by aqueous (9.66±0.57mm). *P. aeruginosa* was found to be more sensitive to the methanol extract of *C. religiosa* with a maximum inhibitory zone (7.33±0.57mm), followed by the least sensitive exhibited to the aqueous extract (6.66±0.57mm).

The anti-microbial activity of aqueous extracts of *C. abbreviate, L. edulis and Z. mucronata* are shown in Table 5. From observation, it is apparent that the plant extracts did not possess any form of anti-bacterial activity against the panel of microorganisms or the bacterial species were resistant to the extracts.

The increasing trend of drug resistant mutations is raising greater health concerns. The majority of people residing in rural based settings are interested in herbal remedies that they perceive as being safe and effective [70]. Such advancements have prompted scientists to investigate the efficacy of indigenous medicinal plants with the hope of discovering potential drug candidates that might eradicate different diseases and overcome the issue of resistance. The type and level of biological activities which might be exhibited by any plant extracts depends on many factors such as the plants' part used, geographical location of the plant, plant drying methods and storage conditions. The efficacy of the extracts can be affected by elevated temperature during extraction steps. Such adversity might affect the level and composition of the secondary metabolites within the extract [71].

Table 4 Antimicrobial activity of *Copaifera religiosa* and *C. Lucanusianus* indicating inhibition zones (mm) ± SD

Microorganisms	*C. religiosa*								*C. Lucanusianus*				Positive control
	Methanol extracts				Aqueous extracts				Aqueous extracts				Gentamicin
	Inhibition zones (mm)												
	200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	
B. cereus	15.66±0.57	14.33±0.57	12.66±0.57	12±0	12.33±2.31	12±1.73	9.33±1.15	6.33±0.57	-	-	-	-	25±1.73
S. aureus	22.66±0.57	25±1.73	23.33±1.52	16±2.0	30±0	33.33±1.52	29±1.73	26±2.64	-	-	-	-	25.66±1.15
E. faecalis	10.66±0.57	9.66±0.57	8.33±1.15	6.33±0.57	9.33±1.52	9.66±0.57	7±0	-	-	-	-	-	15.33±0.57
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	21.33±0.57
P. aeruginosa	7.33±0.57	-	-	-	6.66±0.57	-	-	-	-	-	-	-	21±1.00
S. enterica	-	-	-	-	-	-	-	-	-	-	-	-	19±0
E. cloacae	-	-	-	-	-	-	-	-	-	-	-	-	20±0
S. sonnei	-	-	-	-	-	-	-	-	-	-	-	-	18.33±2.08

(- = No activity)

Concerning the antimicrobial activity of *Copaifera religiosa* and *Costus lucanusianus*, only *Copaifera religiosa* extracts displayed activity against some strains tested. Indeed, *C. religiosa* extracts showed inhibition of *P. aeruginosa, E. faecalis, B. cereus* and *S. aureus*. Methanolic *Copaifera regiosa* extract exhibited the highest inhibition on *S. aureus*. Yet, regarding *C. lucanusianus* extracts which did not possess any antimicrobial in this study Baba and Onanuga [41] demonstrated the inhibitory activity of *C. lucanusianus* leaves on *S aureus*, *P. aeruginosa* and *E. coli* at a concentration of 20 mg/ml. This difference on the results may be due to the plant parts used as in this study we worked with stem instead of leaves, also the mode of extract preparation or the geographical location.

The results of the anti-bacterial activity of *C. abbreviate, L. edulis* and *Z. mucronata* demonstrated no activity against the clinical bacterial strains. These results are in contradiction to findings by Olajuyigbe and Afolayan [72]. The bacterial isolates *E. faecalis, E. coli, K. pneumoniae* were shown to exhibit varied degree of susceptibility with zones of inhibition ranging between 17 and 27 ± 1.0 mm; though the difference in results can be attributed to the extraction solvent used.

In addition, a study published by Samie et al [73] showed that ethanolic extracted compounds from *P. angolensis* inhibited bacterial growth of *Staphylococcus aureus* with median inhibitory concentration of 25 µg/ml.

In this study, methanolic extracts displayed higher anti-microbial activity than the aqueous extracts. The results obtained in this study are not surprising because previous reports have shown that certain plant water extracts do not possess any biological activity [74].

Table 5 Antimicrobial activity of *C. abbreviate, L. edulis and Z. mucronata* indicating inhibition zones (mm) ± SD

Organism	Positive control	MIC (µg/ml)	***C. abbreviata***	***L. edulis***	***Z. mucronata***
Enterococcus faecalis	Vancomycin hydrochloride	2	-	-	-
Escherichia coli	Gentamicin sulfate	8	-	-	-
Klebsiella pneumonia	Gentamicin sulfate	2	-	-	-
Salmonella	Gentamicin sulfate/ imipenem dehydrate/ amicillin/ penicillin G/ chloramphenicol	Resistant	-	-	-
Streptococcus agalactiae	Vancomycin hydrochloride	2	-	-	-

(- = No activity)

3.4 Immunomodulatory effects of plants

Several medicinal plants and their compounds have been recorded to possess immunomodulatory potentials [75].

However, no reports were found that have documented on the potential of *C. abbreviata* as an immunomodulator.

In this review, the aqueous extracts of *C. abbreviate, L. edulis* and *Z. mucronata* were investigated for their effects on the production of IL-2, IL-6 and TNF-α in PBMCs (Table 6). Firstly, the viability of cells was assessed at two concentrations (50 and 150 µg/ml) using the MTT assay. Proliferation of cells was induced with high concentrations of *C. abbraviata* and *L.edulis* extracts. IL-2 production was increased by low concentrations of *C. abbraviata* and *Z. Mucronata* while *L.edulis* extract induced secretion of IL-2 at both concentrations. An increase in IL-6 production was observed for all 3 plants at both concentrations tested. The secretion of TNF-α was induced by *C. abbraviata* only.

Brendler and Van Wyk [76] showed that phenolic compounds have strong immunomodulatory activities. A study done by Gayathri et al [77] have indicated that the ability of plants to suppress immune system response was associated with the presence of some compounds such as glycosides, saponins, flavonoids, and steroids. Most of these compounds were found to be present in both *C. abbreviata* and *L. edulis*. Indeed, plants have been used as immunomodulators in the treatment of various diseases [78] even in the treatment of autoimmune diseases [79,80]. Therefore, medicinal plants able to inhibit excessive immune responses might have useful applications in the treatment of immunological disorders [81].

Table 6 Immunomodulatory effects of aqueous extracts *C. abbreviate, L. edulis* and *Z. mucronata* on PBMCs

Plants tested		Viability (Absorbance)	IL-2 (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
Control	Cells only	0.16	52	133	17
	Positive control (PHA)	0.327	95	488	41
	Negative control (DMSO)	0.02	0	109	16
C. abbraviata	100 µg/ml	0.318	39	179	26
	50 µg/ml	0.13	96	498	21
Z. mucronata	100 µg/ml	0.145	69	350	16
	50 µg/ml	0.135	85	471	15
L. edulis	100 µg/ml	0.244	64	142	13
	50 µg/ml	0.155	107	211	12

4. Conclusion

The present review aimed at highlighting studies done on 5 indigenous plants from Gabon and South Africa and giving the most recent results obtained in house. The literature review indicated the plants to possess a wide range of compounds with known pharmacological properties. These findings were emphasized by the results obtained in house demonstrating their phytochemical composition, immunomodulatory and antimicrobial activities. The compounds found could be used as therapeutic agents in preventing or treating diseases caused by oxidative stress and different pathogenic strains such as *S. aureus, B. cereus, E. faecalis,* and *P. aeruginosa*. In addition, the results obtained, validate traditional healers' claims in treating various infections. However, it should be reported that the remedies made by traditional healers are a composition of various parts of different plant species, in order to improve the efficacy and the quality of the remedies given to people. This study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

Acknowledgements This study is supported by funding from the University of Venda Research Directorate through Dr AN Traoré. We would also like to thank the South African National Research Foundation for providing bursaries to the students.

References

[1] Elumalai P, Gunadharini DN, Senthilkumar K, Banudevi S, Arunkumar R, Benson CS, Sharmila G, Arunakaran J. Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. *Toxicology letters*, 2012; **215:** 131-142.
[2] Yaw VB, Samuel OA, Gyan BA, Ethnobotanical survey of plants used in the treatment of malaria in the Sekyere Central District of Ashanti Region of Ghana. *International Journal of Novel Research in Life Sciences*. 2015; **2:** 17-25.
[3] van Wyk B, Wink M. Medicinal plants of the world. Briza Publications: Pretoria; 2004 ISBN-1-875093443.
[4] World Health Organization (WHO). Traditional medicine. Media site; 2003 [cited April 2017]. Available from: http://www.who.int/mediacentre/factsheets/2003/fs134/en/.
[5] Hassan SE, Hijri M, St-Arnaud M. Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. *New biotechnology*, 2013; **30:** 780-787.
[6] Shakeri A, Hazeri N, Vlizadeh J, Ghasemi A, Tavallaei FZ. Phytochemical screening, Antimicrobial and antioxidant activities of Anabasis aphylla L. extracts. *Kragujevac Journal of Science*, 2012; **34:** 71-78.
[7] Roberts I, Smith S, De Rybel B, Van Den Broeke J, Smet W, De Cokere S, Mispelaere M, De Smet I, Beeckman T. The CEP family in land plants: evolutionary analyses, expression studies, and a role in Arabidopsis shoot development. *Journal of Experimental Botany*. 2013: **64:** 5371-5381.
[8] Hasplova K, Hudecova A, Miadokova E, Magdolenova Z, Galova E, Vaculcikova L, Gregan F, Dusinska MN. Biological activity of plant extract isolated from Papaver rhoeas on human lymfoblastoid cell line. *Neoplasma.* 2011; **58:** 386-91.
[9] Tadeusz A. Alkaloid chemistry, biological significance, applications and ecological role. United Kingdom. Elsevier, 2007; 141-147.
[10] Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*. 2006; **27:** 1-93.
[11] Farnsworth NR. 1994. Ethnopharmacology and drug development, Ethnobotany, drug development and biodiversity conservation-exploring the linkages. In: *Ethnobotany and the Search for New Drugs,* Ciba Foundation Symposium, vol. 185. John Wiley and Sons, Chichester, pp. 42–59.
[12] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. 2001; **109:** 69–75.
[13] Nakweti RK, Franche C, Ndiku SL. Effects of Sodium Azide (NaN3) on Seeds Germination, Plantlets Growth and In vitro Antimalarial Activities of Phyllanthus odontadenius Müll. Arg. *American Journal of Experimental Agriculture*, 2015; **5:** 226.
[14] Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal plants. *Tropical Horticulture*, 1998; **2:** 449-632.
[15] National Reference Centre for African Traditional Medicines (NRCATM). 2004. A South African Model, Department of Health (DOH), Medical Research Council (MRC), Council for Scientific and Industrial Research (CSIR). Available Online: http://www.sahealthinfo.org/traditionalmeds/traditionalpartl.pdf. Accessed 01 February 2016.

[16] Scott G, Springfield EP. *Pharmaceutical monographs of 60 indigenous plant species used as traditional medicines in South Africa*, 2004; South African National Biodiversity Institute (SANBI) plant information website at www.plantzafrica.com.
[17] van Wyk BE, Gericke N. People's Plants: A Guide to Useful Plants of Southern Africa. Briza Publications: Pretoria; 2000.
[18] Louw CAM, Regnier TC, Korster L. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. *Journal of Ethnopharmacology.* 2002; **82:** 147 – 154
[19] Bessong PO, Obi CL, Andreola M, Rojas LB, Pouysegu L, Igumbor E, Meyer JJM, Quideau S, Litvak S. Evaluation of selected South African medicinal plants for inhibitory properties against immunodeficiency virus type 1 reverse transcriptase and integrase. *Journal of Ethnopharmacology*. 2005; **99**: 83 - 91.
[20] Samie A, Obi CL, Bessong PO, Namrita L. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African journal of Biotechnology*, 2005; **4:** 1443-1451.
[21] Bessong PO, Obi CL, Andreola ML, Igumbor E, Litvak S. *In vitro* activity of three selected South African medicinal plants against human immunodeficiency virus type 1 reverse transcriptase. *African Journal of Biotechnology*. 2004; **3:** 555-559.
[22] Mabogo D EN. The ethnobotany of the Vhavenda. M.Sc. Thesis; 1990. University of Pretoria; Pretoria, South Africa.
[23] Mills EJ, Foster BC, van Heeswijk RP, Wilson KEP, Kosuge K, Leonard B, Kanfer I. Impact of African herbal medicines on antiretroviral metabolism. *AIDS.* 2005; **19:** 95-7.
[24] Mulaudzi RB, Ndhala AR, Kulkarni MG, Finnie JF, Van Staden J. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. *Journal of Ethnopharmacology.* 2011; **135:** 330-337.
[25] Tshikalange TE, Meyer JJ, Hussein AA. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. *Journal of Ethnopharmacology*. 2005; **96:** 515-519.
[26] Madzibane J, Potgieter MJ. Uses of *Colophospermum mopane* (Leguminasae: Caesalpiniodeae) by the VhaVenda. *South African Journal of Botany*. 1999; **66:** 112-114.
[27] Brendt HD, Muller GJ. Traditional medicines and acute poisoning. *Continuing Medical Education.* 1995; **13:** 1053-1060.
[28] Steenkamp V. Phytomedicines for the prostate. Fitoterapia. 2003; **74:** 545-52.
[29] Yirga G. Assessment of indigenous knowledge of medicinal plants in central zone of Tigrayi, Northern Ethiopia. *African Journal of Plant Science*. 2010; **4:** 006-011.
[30] Bessong P., Rojas LB, Obi LC, Tshisikawe PM, Igunbor EO. Further screening of Venda medicinal plants for activity against HIV type 1 reverse transcriptase and integrase. *African Journal of Biotechnology*, 2006; **5:** 526-528.
[31] Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. Champman and Hall, London, 1998.
[32] Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific Journal of Tropical Biomedicine*, 2013; **3:** 705-710.
[33] Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exp.* 1990; **15:** 113-115.
[34] Ngcobo M, Nkala B, Moodley I, Gqaleni N. Recommendations for the development of regulatory guidelines for registration of traditional medicines in South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 2012; **9:** 59-66.
[35] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods.* 1983; **65:** 55-63.
[36] De La Estrella M, Cabezas FJ, Aedo C, Velayos M. Checklist of the Caesalpinioideae (Leguminosae) of Equatorial Guinea (Annobón, Bioko and Río Muni). *Botanical Journal of the Linnean Society*, 2006; **151:** 541-562.
[37] Brink K, van Vuren J, Bornman R. Responses of laboratory exposed catfish (Clarias gariepinus) to environmentally relevant concentrations of p, p′-DDT. *Environmental toxicology and pharmacology*, 2012; **34:** 919-925.
[38] Lekana-Douki JB, Liabagui SLO, Bongui JB, Zatra R, Lebibi J, Toure-Ndouo FS. In vitro antiplasmodial activity of crude extracts of Tetrapleura tetraptera and Copaifera religiosa. *BMC research notes*, 2011; **4:** 506.
[39] Kay KM, Reeves PA, Olmstead RG, Schemske DW. Rapid speciation and the evolution of hummingbird pollination in neotropical Costus subgenus Costus (Costaceae): evidence from nrDNA ITS and ETS sequences. *American Journal of* Botany, 2005; **92:** 1899–1910.
[40] Salzman S, Driscoll HE, Renner T, André T, Shen S, Specht CD. Spiraling into history: A molecular phylogeny and investigation of biogeographic origins and floral evolution for the genus Costus. *Systematic botany*, 2015; **40:** 104-115.
[41] Baba H, Onanuga A. Preliminary phytochemical screening and antimicrobial evaluation of three medicinal plants used in Nigeria. African Journal of Traditional, Complementary and Alternative Medicines, 2011; **8:** 387-390
[42] Owolabi, OJ, Omogbai EKI, Obasuyi O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. Afr. J. Biotechnol. 2007; **6:** 882-85.
[43] Betti JL, Yongo OD, Mbomio DO, Iponga DM, Ngoye A. An ethnobotanical and floristical study of medicinal plants among the Baka Pygmies in the periphery of the Ipassa-Biosphere reserve, Gabon. *European Journal of Medicinal Plants*, 2013; **3:** 174.
[44] Termote C. 2012. Wild edible plant use in Tshopo District, Democratic Republic of Congo (*Doctoral dissertation, Ghent University*), 2012; pp 80.
[45] Vliet EV. Medicinal plants for women's en children's health in urban and rural areas of Gabon. Gabon. Major research project,master Environmental Biology, Utrecht University. 2014; pp. 14-15.
[46] Aweke G. 2007. Costus afer Ker Gawl. Record from PROTA4U. *PROTA (Plant Resources of Tropical Africa/Ressourcesvégétales del'Afrique tropicale). The Netherlands: Wageningen. Available from: http://www. prota4u. org/search. asp [last accessed 16 Apr 2014]*.
[47] Owolabi OJOE, Falodun A. Oxytocic effects of the aqueous leaf extract of Costus lucanusianus - family Costaceae on isolated non-pregnant rat uterus. Pak J Pharm Sci. 2010; **23**:207–211.
[48] Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research*, 2010; **4:** 191-195.
[49] Venter F, Venter J. Making the most of indigenous trees, Second edition. Fourth Impression, Briza Publications, Pretoria, South Africa. 2009; pp 92.
[50] Coates Palgrave M. 2005. Keith Coates Palgrave Trees of Southern Africa. imp. 3.
[51] Setshogo MP, Mbereki CM. Floristic diversity and uses of medicinal plants sold by street vendors in Gaborone, Botswana, 2011.
[52] Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi ENM. Traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast. *Journal of ethnopharmacology*, 2007; **114:** 377-386.

[53] Makundi EM, Malebo HM, Mhame P, Kitua AY, Warsame M. Role of traditional healers in the management of severe malaria among children below five years of age: the case of Kilosa and Handeni Districts, Tanzania. *Malaria Journal*, 2006; 5: 58.
[54] Ribeiro A, Romeiras MM, Tavares J, Faria MT. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. *Journal of ethnobiology and ethnomedicine*, 2010; **6:** 33.
[55] Bruschi P, Morganti M., Mancini M, Signorini MA. Traditional healers and laypeople: a qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). *Journal of Ethnopharmacology*, 2011; **138:** 543-563.
[56] Kisangau DP, Herrmann TM, Lyaru H, Hosea K, Joseph C, Mbwambo Z, Masimba P. Traditional knowledge, use practices and conservation of medicinal plants for HIV/AIDS care in rural Tanzania. *Ethnobotany Research and Applications*, 2011; **9:** 043-057.
[57] Mongolo NI, Mafoko BJ. *Cassia abbreviata* Oliv. A review of its ethnomedicinal uses, toxicity, phytochemistry, possible propagation techniques and pharmacology. *African Journal of Pharmacy and Pharmacology*, 2013; **7:** 2901–2906.

[58] Moshi MJ, van den Beukel CJ, Hamza OJ, Mbwambo ZH, Nondo RO, Masimba PJ, Matee MI, Kapingu MC, Mikx F, Verweije PJ, van der Ven AJ. Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *African Journal of Traditional, Complementary and Alternative Medicines*, 2007; **4:** 219-225.
[59] Leteane MM, Ngwenya BN, Muzila M, Namushe A, Mwinga J, Musonda R, Moyo S, Mengestu YB, Abegaz BM, Andrae-Marobela K. Old plant newly discovered: *Cassia sieberiana* D.C and *Cassia abbreviata* Oliv, Root extracts inhibit *in vitro* HIV-12c replication in peripheral mononuclear cells (PBMCs) by different modes of action. *Journal of Ethnopharmacology*. 2002; **141:** 48-56.
[60] Sigrid Leger. The Hidden Gifts of Nature, Forsthaus 1, 2000-2003; D-85290, Germany.
[61] Watt JM, Breyer-Brandwick MG. The Medicinal and poisonous plants of Southern and Eastern Africa, 2nd ed. 1962; E&S Livingstone, London, p. 745.
[62] Elgorashi EE, Taylor JL, Maes A, van Staden J, De Kimpe N, Verschaeve L. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicology letters*, 2003; **143:** 195-207.
[63] Aganga AA, Adogla-Bessa T. Dry matter degradation, and tannin and crude protein contents of some indigenous browser plants of Botswana. *Archives of zootechnology*, 1999; **48:** 79-82.
[64] Gelfand M, Mavi M, Drummond RB, Ndemera N, 1985. Traditional medical practitioners in Zimbabwe. Mambo press Gweru pages; 1985; **23:** 57-75.
[65] Queiroz EF, Kuhl C, Terreaux C, Mavi S, Hostettmann K. New dihyroalkylhexenones from *Lannea edulis*. *Journal of Natural Products*, 2003; **66:** 578-580
[66] Sohni YR, Davis CL, DesChamps AB, Kale PG. Frameshift mutations in Salmonella induced by the extracts of medicinal herb Lannea edulis (Sond.) Engl and Monotes glaber Sprague. *Environmental and Molecular Mutagenesis*, 1995; **25:** 77-82.
[67] Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of medicinal plants research*, 2011; **5:** 6697-6703.
[68] Abubakar MG, Vicwvani AN, Shehu RA. Phytochemical screening of antibacterial activity of *Tamarinduc Indica* pulp extract. *Asian Journal of Biochemistry*. 2008; **3:** 134 – 138.
[69] Ngbede J, Yakubu RA, Nyam DA. Phytochemical screening for active compounds in *Canarium, Schweinfurthii* (Atile) leaves from Jos North, Plateau State Nigeria. *Research Journal of Biological Sciences*. 2008; **3:** 1076 – 1078.
[70] Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci. 2005; **78:** 431-41.
[71] Wendakoon C, Calderon P, Gagnon D. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *Journal of Medicinally Active Plants*. 2012; **1:** 60-68.
[72] Olajuyigbe OO, Afolayan AJ. In vitro ethnotherapeutic potential of acetone extract of the bark of *Ziziphus mucronata* Willd.subsp. *mucronata* Willd: Antimicrobial and toxicity evaluations. *African Journal of Biotechnology.* 2012; **11:** 16783-16789.
[73] Samie A, Housein A, Lall N, Meyer JJM. Crude extracts of, and purified compounds from *Pterocarpus angolensis*, and the essential oil Lippia javanica: their *in vitro* cytotoxicities and activities against selected bacteria and *Entamoeba histolytica. Annals of Tropical Medicine and Parasitology.* 2009; **133:** 427 – 439.
[74] Luseba D, Van Staden J, Elgorashi EE, Nthloedibe DT. Antibacterial, anti-inflammatory and cyto-toxicity study of medicinal plants used in treatment of wounds and retained placenta in animals. *South African Journal of Botany.* 2007; **73:** 378-383.
[75] Bin-Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I, Raisuddin S. Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. *International immunopharmacology*, 2003; **3:** 257-265.
[76] Brendler T, van Wyk BE. A historical, scientific and commercial perspective on the medicinal use of Pelargonium sidoides (Geraniaceae). *Journal of ethnopharmacology*, 2008; **119:** 420-433.
[77] Gayathri V, Asha VV, Subramoniam A. Preliminary studies on the immunomodulatory and antioxidant properties of *Selaginella* species. *Indian J Pharmacol,* 2005; **37**: 381–385.
[78] Boehm K, Bussing A, Ostermann T. Aromatherapy as an adjuvant treatment in cancer care: A descriptive systematic review. *Afr. J. Traditional Complementary Altern. Med.*, 2012; **9:** 503-518.
[79] Murakami M, Hyakusoku H, Ogawa R. The use of Japanese "kenzan" flower holders to create scar-less drainage holes in skin grafts. *Burns*. 2010; **36:** 732-733.
[80] Ramgolam V, Ang SG, Lai YH, Loh CS, Yap HK. Traditional Chinese medicines as immunosuppressive agents. *Annals of the Academy of Medicine, Singapore*, 2000; **29:** 11-16.
[81] Mirshafiey A, Khorramizadeh M., Saada, F, Rehm BH. Chemopreventive effect of M2000, a new anti-inflammatory agent. *Medical Science Monitor*, 2004; **10:** PI105-PI109.