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Artemisia afra and Modern Diseases

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Abstract

Herb *Artemisia afra* has recently attracted worldwide attention of researchers for its possible use in the modern diseases like diabetes, cardiovascular diseases, cancer, respiratory diseases etc. This review is exhaustive and systematic organization of the available literature on *Artemisia afra (A. afra)* from January 1922 to July 2011. The literature survey presents the number of publications with respect to time. Patents are briefly described; the traditional uses are classified and summarized. Some emphasis is given to the data and projections of modern diseases and the ongoing research in this area in the context of title of this review. The pharmacognostic aspects, chemical constituents and factors affecting it, the activity, analysis & quality control, pharmaceutical dosage form etc. is dealt in this review.

Keywords: *Artemisia afra*; Patents; Traditional uses; Chemical constituents; Activity; Toxicity; Dosage form

Introduction

Man has been able to appreciate through his superior observing and learning capabilities to use and exploit the natural resources, the flora and fauna for his survival and comfort, to alleviate pain and to cure diseases; to constantly improve upon his health and build longevity. WHO [1] reports that 80% population of Asian and African countries depend on traditional medicine to treat various infectious and chronic disease conditions. The popularity of this system of medicine is due to people's faith in traditional age old methods, its accessibility and affordability [2-4].

South Africa is considered the "hotspot" for its unique and diverse botanical heritage [5,6]. Recent statistics show that about 25% of the total number of higher plants in the world are found in South Africa [7] although its land surface make up less than 1% of the earth [5]. According to the "African Plant Checklist and Database Project" [8], a total of 50,136 angiosperm taxa occur in tropical and southern Africa. It is estimated [5,9] that about 3000 medicinal plants are used in South Africa by traditional healers with an estimated 27 million consumers [10]. Of these, only 350 species are most commonly used and about 38 indigenous species have been commercialized to some extent (i.e. they are available as processed materials in modern packaging and in various dosage forms as teas, tinctures, tablets, capsules or ointments). Very few medicinal plants are studied for their potential therapeutic properties [11]. Several others are also produced for multi-million Rand informal markets [12-14]. Basic information about most widely used species can be found in van Wyk et al. [6,9] and Diederichs [15]. It is generally accepted [16] that natural resources will play a major role in the socioeconomic development of the African continent. It is found that herbal treatments are highly lucrative in the international market. The annual revenues in Western Europe reached US $5 billion in 2003-04, in China sales of products totaled US $14 billion in 2005 and herbal medicine revenue in Brazil was US $160 million in 2007 [1] and involves qualified traditional healers, as well as thousands of commercial gatherers who supply both the formal and informal entrepreneurial sectors of the South-African economy [17-22].

A. afra is one of the important and most widely used herbs in the traditional medicine. In recent years, it has gained significant attention from the scientific community. Studies have been conducted either to verify or substantiate the traditional use of this herb. Further, its use is also being investigated in the modern diseases like diabetes, cardiovascular diseases, cancer, respiratory diseases etc. With the quantum of work going around and the various properties that are being studied, it was felt to undertake an exhaustive literature survey of this herb *A. afra* from South Africa, and scientifically compile the information in a comprehensive review.

Literature Search

The number of hits on the internet based science-specific search engine "Scirus" [23] up to mid July 2011 for the key words and the details thereof are given in Table 1. The significance of genus *Artemisia* is seen in its number of hits, which is 89,080.

The total number of hits appeared for "*Artemisia afra*" (*A. afra*) were 885 of which, 5 had no dates. Figure 1 is the graph of 162 publications that appeared in Journal Sources classified and plotted on yearly basis from Jan. 1922 to Nov. 2011 for "*A. afra*".

Only two scientific publications based on laboratory work were found in the literature over a span of half a century, first by Goodson in Jan. 1922 [24] and then second by Bohlman and Zdero in 1972 [25]. Both the papers report the constituent's of *A. afra.* Goodson investigated if *A. afra* contained anything that could be regarded as a precursor or a derivative of santonin in consequence of the difficulty of obtaining santonin that was then used as the sole source of anthelmintic. He showed that *A. afra* contains camphor, a wax–ester probably ceryl cerotate, triacontane, scopoletin and quebrachitol and none which

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Details	*Artemisia*	*A. afra*	*A. afra* and					
			Activity	Toxicity	Cancer	Diabetes	Cardiovascular Disease	Respiratory Disease
Total Hits	89,080	885	748	160	164	97	16	6
Period of Publication	Dec. 1884 to Sept. 2013	Jan. 1922 to Nov. 2011	Mar. 1961 to Nov. 2011	Jan. 1980 to Jun. 2011	Jan. 1990 to Nov. 2011	Jan. 2002 to Nov. 2011	Jan. 2005 to Apr. 2011	Jan. 2005 to Apr. 2011
Journal Sources	12,318	163	128	67	49	31	3	-
Preferred web	7,172	454	451	19	17	18	5	1
Other web	3,806	268	169	74	98	48	8	5

Table 1: Total hits for Artemisia, publication period and the number of publications.

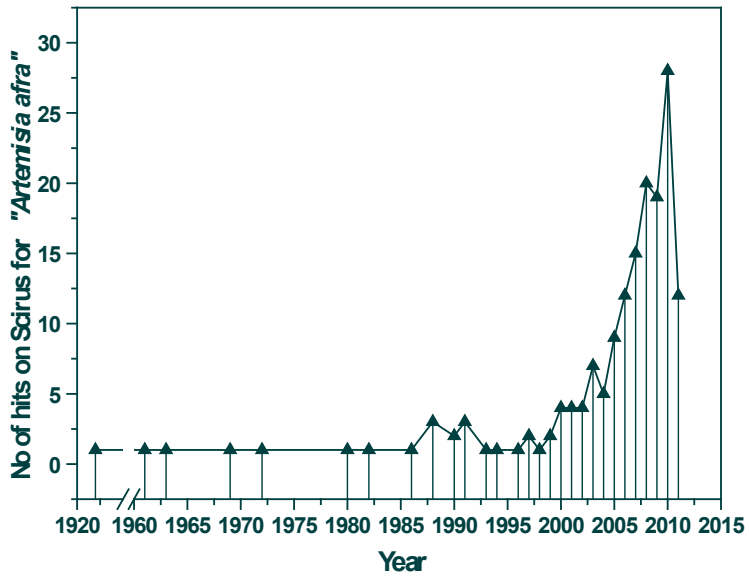

Figure 1: Number of hits for *Artemisia afra* on Scirus, Science specific search engine on year basis.

could be connected to santonin. While, Bohlman and Zdero [25] investigated to compare other constituents in *A. afra* with those of the old world *Artemisia* species and reported that the roots contained besides isomeric coumarins (mainly (VI)) the known acytylenes (I to V) while the aerial parts contained thujone and umbelliferone-derivatives and no acetylenes (Figure 2). However, Jakupovic et al team [26] of researchers reported additionally 10 new guaianolides and 5 glucolides and also 12-hydroxy-α-cyperone elucidated by high field NMR techniques and some chemical transformations. It can be said that upto 1988, scientific curiosity was generally confined in determining the constituents of *A. afra* essential oil and assigning chemical structure to it.

From Figure 1, it can be said that up to year 2000, *A. afra* did not attract the researchers but only later, especially from 2005 onwards with an average of 18 publications per year. The University of Western Cape and Rhodes University, South Africa is doing lot of research on this plant. The scientific studies made on the plant extracts/essential oil from 1993 onwards were in the direction of finding out the activity namely antifungal [27], antibacterial [28], antioxidant [29], toxicity [30], anti-cancer [31], antituberculotic [32], antimalarial [33] antitrypanosomal [34], protective myocardial [35], protective intestinal epithelial Caco-2 cells [36], anti-ulcerative [37] effect on Central Nervous System [38] etc. Pharmaceutical efforts were also made to prepare tea bags from the leaves of *A. afra* [39] to liposomal encapsulation of the essential oil [40] for clinical trials. The traditional claims were also scientifically investigated especially for gynecological complaints [4] and in respiratory disorders [41]. The veterinary anti-oxidant use in attenuating coccidoss in broiler chicken was also explored by Naidoo [42].

The quantitative estimations of plant extracts by using modern equipments like GCMS and GC; HPLC and UV absorption and Mass Spectroscopy was taken up by Oyedeji et al. [43] and Avula et al. [44] respectively in 2009. In 1997, the first European patent was granted to Whittle and Skett [45] that relates to administration of compounds for use in the treatment of diabetes and in 2008, two patents viz. Omer [46] and Bobotas et al. [47] were granted. Details are given under the headings "Omer patent to counteract weight loss & treat other diseases in cancer patients" and "Bobotas et al patent for diabetes and cardio-vascular diseases" of this review. Jager et al's [48] patent came in 2009. The literature search [23] for *A. afra* along with the additional key words of pharmacological importance and the number of hits are compiled in Table 1.

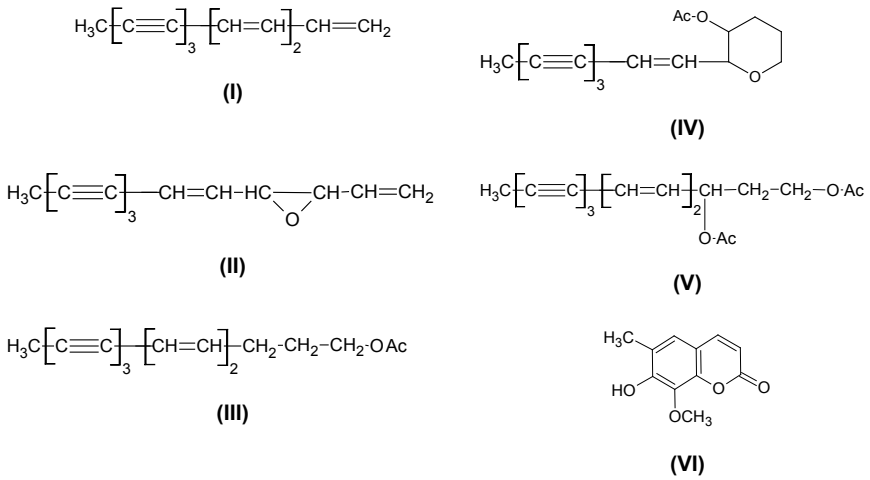

Figure 2: Constituents of A. afra. Reprinted from Phytochemistry, 11(7), F. Bohlmann, C. Zdero, Constituents of Artemisia afra, 2329-2330, 1972 with permission from Elsevier [25].

A pubmed data base [49] search till 13th July 2010 with the search key word "Artemisia" contained 1851 hits of which 91 were reviews, and for "*A. afra*" only 24 articles of which 1 was a review entitled "A broad review of commercially important southern African medicinal plants" by van Wyk in Oct. 2008 issue of Journal of Ethanopharmacology [7]. With this background of *A. afra* gaining importance and an objective to compile a comprehensive review with the available scientific information in one paper, this review is being written.

Patents

There are four utility patents given for *A. afra* from Oct. 1997 till July 2011. Two patents each were granted in US and in Europe. Two patents mainly are in diabetes, one in cancer and; one in diabetes and cardiovascular disease. The gist of all the four patents is given below in reverse chronological order. It is expected to give readers some clues for further research and application.

Jager et al patent to prevent/ treat diabetes and associated secondary diseases

The Jager et al. [48] patent relates to a physiologically active composition in pure or mixture form containing *Artemisia* extract of at least one of *A. dracunculus*, *A. herba-alba*, *A. judaica*, *A. vulgaris*, *A. abysinica*, *A. absynthicum*, *A. afra, A. cannariensis*, *A. pallens*, *A. annua*, *A. abrotanum*, *A. ludoviciana*, *A. capillaris* or *A. scoparia* to prevent or treat (pre)diabetes and associated accompanying diseases or secondary diseases. The patent claims that at least one of following could happen: (a) the blood sugar level in a mammal would be lowered, (b) the insulin resistance would be lowered, (c) hepatic glucose release would be lowered, (d) the postprandial glucose level would be lowered, (e) the activity of GLP-1 ("glucagon-like-peptide 1") would be raised, (f) the binding capacity between GLP-1 and the associated receptor would be raised, (g) the conversion of glucose to glycogen would be raised, (h) the expression of the IRS-2 ("insulin receptor substrate 2") polypeptide would be raised, and (i) the insulin-controlled glucose uptake would be raised. The composition can be given as a food supplement, a drink, a food, a dietetic food, a functional food or a sport food wherein the effective daily amount of the composition would be between 0.1 and 500 mg/kg/daily with respect to bodyweight.

Omer patent to counteract weight loss & treat other diseases in cancer patients

The patent assigned solely to Omer [46] based on clinical trial claims to counteract the weight loss and nutritional deficiency of cancer patients, and to treat Hodgkin and Non-Hodgkin lymphomas, autoimmune diseases, IgA-Nephropathy (glomerulonephritis) and human cancers with a herbal preparation containing *Artemisia*. The object of the patent is to (a) circumvent resistance to conventional chemotherapy of these diseases, (b) increase the effectiveness of chemotherapy when added to standard chemotherapy treatment, (c) provide treatment to those cancer patients and IgA Nephropathy where no effective treatment is available so far, (d) improve nutrition of patients suffering from progressing cancer, as all cancer patients start losing weight at some stage of their disease, (e) provide a composition aforesaid that acts without exerting toxic side effects and (f) provide a disease-specific synergistic composition in convenient dosage form. The preparation could be of any species from *Artemisia* viz., *A. absinthium*, *A. annua*, *A. vulgaris* and *A. capillaris* or any bitter or aromatic herb or shrub of the genus *Artemisia* of the family *Asteraceae*, distributed throughout many parts of the world; which shall also contain 10-80% by weight of ginger root (*Zingiber officinale* rhizomes) and 10-80% by weight of large cardamom (*Amomum subulatum*). The amount of herbs in the dose could be sufficient to suppress the progress of the disease. The preparation could be prepared either by grinding (filling the powder in hard gelatin capsules or pressing them in tablets), solvent extraction or distillation (tincture), which may also contain an inert pharmaceutical carrier. This preparation could be used in combination with conventional standard therapy to modulate the immune system of the human body. The dose for various forms could be: (a) Dried Herbal Powder: 3-6 tablets per day as 750 mg pressed tablets, (b) Liquid Extract: 2 ml three times a day (equivalent to 6 gm of herb), (c) Distilled Preparations: 2 ml three times a day (equivalent to 6 gm of herb) and (d) Tincture (powdered herbal extracts): 6-9 capsules per day as 450 mg capsules of mixtures.

Bobotas et al patent for diabetes and cardio-vascular diseases

The patent assigned to Bobotas et al. [47] relates to methods of using different compositions comprising a (i) cannabinoid 1 (CB1) antagonist, (ii) a dyslipidemic agent, and/or (iii) a metabolic regulator

useful in treating hypertriglyceridemia, hypercholesteremia, mixed dyslipidemia, vascular disease, artherosclerotic disease, and/or obesity; preventing and/or reducing cardiovascular and/or vascular events; reducing insulin resistance, fasting glucose levels, and/or postprandial glucose levels; and preventing and/or reducing the incidence of and/or delaying the onset of metabolic syndrome. The preferred dyslipidemic agents could be omega-3 fatty acids, peroxisome proliferator-activated receptor (PPAR) agonists/antagonists, microsomal triglyceride transfer protein (MTP) inhibitors, and/or dipeptidyl peptidase-4 (DPP4) inhibitors; and the preferred metabolic regulators could be sarsasapogenin, smilagenin, steroidal glycosides and extracts thereof, and extracts of *Artemisia spp*.; either alone or in combinations with CB1. The dosage form could be a tablet, hard/soft gelatin capsule, powder that could be dispersed in a beverage, liquid or infusion for oral use, and injectables. The dosage can vary from 1-10 units depending on the combination and requirement.

Whittle and skett pharmaceutical patent in diabetes and hyperglycemia

The Whittle and Skett patent [45] relates to administration of pharmaceutical compounds and compositions for use in the treatment of diabetes or other hyperglycaemic defects of carbohydrate metabolism which includes extracts from plant *Artemisia* spp, (*A. herba-alba*, *A. pallens* or *A. afra*, *A. judaica*), so prepared that it contains effective ingredients viz., insulinomimetic and substance having glucagon antagonist properties with acceptable excipient which can be given orally or by parentral route. The patent also relates how the manufacture method of successive fractionation of the plant extract improves its utility by reducing its toxicity and yielding insulin-like and glucagons antagonist activities thereby increasing therapeutic efficacy. To achieve this, the alcoholic extract obtained by (i) extracting the plant with water, (ii) concentrating the extract to dryness, and (iii) treating the dry residue with alcohol with successive chromatographic separation with different mobile phases (i.e., gradient of eluents) and lastly (iv) the eluate containing at least one portion of the effective ingredient is selected from the different portions related to different mobile phases.

Traditional Uses

A. afra belonging to genus Artemisia is exceptionally and widely used in many parts of the world either alone or in combination with other plants as herbal remedies for a varietyof ailments like simple headache to neurological disorder like epilepsy. There are more than 1 lakh traditional healers practicing in South Africa [13]. In this section, the various conditions in which *A. afra* is being traditionally used, as cited in the literature is given.

Respiratory tract related problems

It is primarily used in common cold, cough, sore throat, influenza, asthma as it is said to clear the respiratory and bronchial passages [50-53]. The leaves are heated and the vapors inhaled to alleviate symptoms of colds and flu [52,54]. It is also used to clear the blocked nasal passage by inserting fresh leaves in the nostrils or by using as snuff; to relieve pain in the throat in scarlet fever, either the hot infusion is used as gargle or the throat is exposed to vapors [52,55]. The leaves are commonly smoked by some tribes to help release phlegm, to ease and soothe a sore throat, coughing at night [56]. For cold and chest problems in infants, fresh leaves are placed in flannel bag and hung around baby's neck [57]. The use of *A. afra* in combination with other medicinal plants has been widely documented in the ethnobotanical literature is given [58] in Table 2.

Gastro intestinal disorders

It is used in the digestive complaints like indigestion, colic, constipation, flatulence, gastritis, dry dyspepsia and to get rid of intestinal worms [51,59-61]. It is consumed to overcome general debility and as an appetizer [62-64]. The leaves are prepared as an infusion or decoction and taken orally.

Topical use for skin afflictions

Watt and Breyer-Brandwijk [52] report that the (i) extract is applied

Plant in combinations	Uses	Administration
A. afra and *E. globulus*	Respiratory complaints	Crushed leaves or steam from infusions are inhaled or decoctions are taken
A. afra and *A. betulina*	Respiratory complaints	Herbal wine
A. afra and *Zanthoxylum capense*	The Europeans and Africans use it in febrile conditions, and it is used as a treatment for colds	A decoction and an infusion of the leaf is used
A. afra and *O. asteriscoides*	Respiratory complaints	Tincture
A. afra, E. globulus and *Leonotis microphylla*	Fever, chest infections and digestive disturbances	Infusion
A. afra, Z. capense and *Allium sativum*	Respiratory complaints	Decoction
A. afra and *Lippia javanica*	Fevers, respiratory complaints, measles and as a prophylactic against lung inflammations	Infusion, taken with milk
A. afra, O. asteriscoides and *E. globulus*	Respiratory complaints	Infusion, tincture
A. afra and *Tetradenia riparia* and salt	Coughs	Decoctions
A. afra and *Alepidea amatymbica*	Colds and flu	Leaves and root/rhizome
A. afra and *Warburgia salutaris*	Acute bronchitis, coughs from colds or flu, fever	Leaves and bark
A. afra, *A. amatymbica* and *Leonotic leonurus.*	Asthma	Leaves and root
A. afra, *W. salutaris* and *Acorus calamus*	Chronic bronchitis and emphysema	Leaves, bark and rhizome

Reprinted from South African Journal of Botany, 76(4), S. Suliman, S. F. van Vuuren, A. M. Viljoen, Validating the in vitro antimicrobial activity of Artemisia afra in polyherbal combinations to treat respiratory infections, 655-661, Copyright 2010, with permission from Elsevier [58].

Table 2: Traditional use of A. afra in combination with other plant species for the treatment of respiratory complaints.

topically to ease the pain and hasten bursting of boils, carbuncles, large acne pimples; (ii) hot bath in the decoction is used to bring out the rash in measles, mumps, chicken pox; (iii) infusion or decoction is used to bathe hemorrhoids, herpes, venereal sores; (iv) poultice (of the leaf) is applied as a dressing to relieve neuralgia, to the swelling of mumps and other glandular or skin inflammations and (v) lotion is used to wash the body to rejuvenate the skin.

Gynecological problems

It is used for dysmenorrhea [9], amenorrhea and menstrual cramps [4]. The genitalia are steamed with vapors for menstrual chills and also after childbirth, while decoctions of leaves have been administered for extended labor [65].

Fever

The decoction of garlic leaves and bulbs is mixed with *A. afra* and *Xanthoxylum capensis* and used as febrifuge [52], a decoction of the plant is drunk as a remedy for fever [66]. An infusion of *A. afra* is widely used in Malaria [66] along with *Lippia javanica* [52]. Bally [67] reports that a poultice is applied on inflamed throat and for fever in children.

Miscellaneous uses

An infusion of a double handful of leaves with a quart of hot water is administered either as enema or emetic for febrile complaints [52]. It is also used in the inflammatory disease like rheumatism [29], gout [51]; neurological disorder like epilepsy [68], in haematuria and to alleviate stabbing pain [69] and as anti-fertility agent [70]. According to Watt and Beyer-Brandwijk [52], *A. afra* has been used to keep urine free from sugar in the case of diabetes mellitus, reports Deutschländer et al. [71], while it is used in tinea captitis reports Abebe and Ayehu [69].

Scope of *A. afra* in Modern Diseases

In the present mechanized life of less physical activity and more mental work, it is pertinent to discuss the afflictions of modern mankind that would also be carried over to new generations, in the context of ongoing global research on the herb *A. afra.*

WHO [72] states that aging of populations in low- and middle–income countries will result in significant increase in the total deaths mostly from non-communicable diseases (NCDs) over the next 25 years, which would be mainly from cardiovascular diseases (CVD), cancers, diabetes and chronic respiratory diseases, causing an estimated 35 million deaths each year. It reports that in year 2004, ~58.8 million deaths occurred globally, of which ~27.7 million were of females and ~31.1 millions of males. More than half of all deaths involved people 60 years and older, of whom 22 million were people aged 70 years and older and 10.7 million were people aged 80 years and older; that almost one in five deaths in the world was of a child under the age of five year. Figure 3 shows the distribution of deaths at all ages for 12 major causes, illustrating the relative importance of the respective causes of death and of male-female differences, in year 2004. WHO projections of Global Burden of Disease (GBD) by cause for 2008, 2015 and 2030 are given in Table 3, based on 2004 GBD estimates as a starting-point [72].

Diabetes

Diabetes is a life threatening condition. The number of people getting affected by diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and mechanized life style. The studies undertaken by Roglic et al. [73] reveals that diabetes is the fifth leading cause of death, killing ~2.9 million people in the year 2000, which is equivalent to 5.2% of all deaths. The prevalence of diabetes and the number of people of all ages with diabetes in the year 2000 and projections for 2030 was estimated by Wild et al. [74]. The paper reports that the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than in women, but there are more women with diabetes than men.

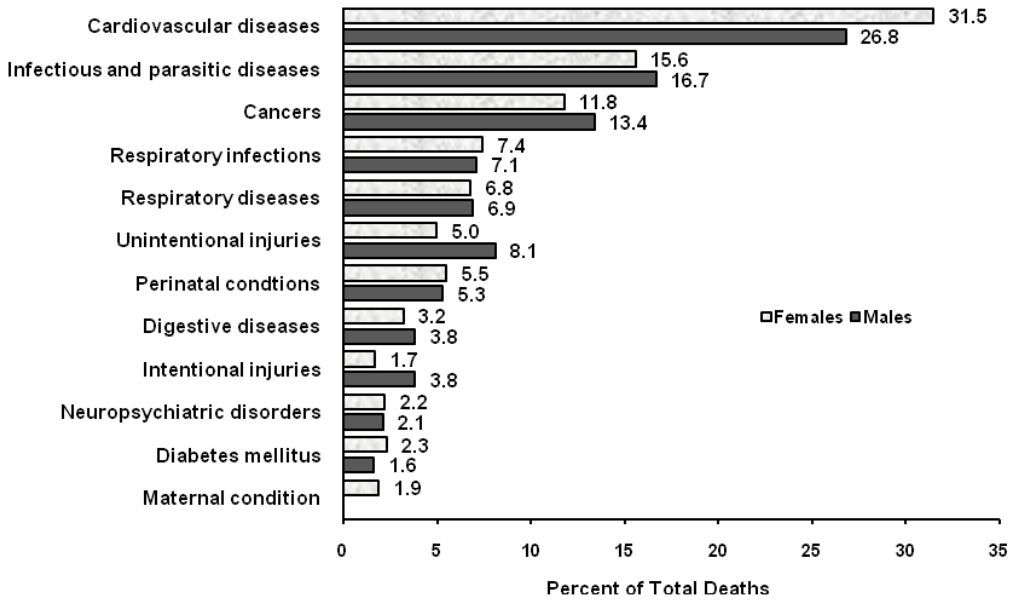

Figure 3: Distribution of deaths by leading cause groups, males and females, world, 2004 [72].

Year	2008		2015		2030	
Population (in billion)	6700628		7186888		8110599	
Disease	**(In 000s)**	**% Total**	**(In 000s)**	**% Total**	**(In 000s)**	**% Total**
Total Deaths	58766	100.0	60856	100.0	67790	100.0
I. Communicable diseases, maternal and perinatal conditions and nutritional deficiencies	**15980**	**27.2**	**13705**	**22.5**	**9370**	**13.8**
Infectious and parasitic diseases (likeTuberculosis, STDs, HIV/AIDS, diarrhea, Meningitis, Hepatitis, Malaria, Trypanosomiasis etc.)	8427	14.3	7044	11.6	4216	6.2
Respiratory infections	3816	6.5	3427	5.6	2871	4.2
Maternal conditions	424	0.7	316	0.5	180	0.3
Perinatal conditions (e)	2913	5.0	2606	4.3	1898	2.8
Nutritional deficiencies	401	0.7	313	0.5	205	0.3
II. Noncommunicable conditions	**37124**	**63.2**	**41193**	**67.7**	**51619**	**76.1**
Malignant neoplasms (like cancer of mouth & orophyrynx, oesophagus, stomach, colon/rectum, liver, pancreas, trachea/bronchus/lung, melanoma & skin, breast, cervix cancer uteri, corpus uteri, prostate, bladder, lymphomas, multiple myeloma, leukaemia etc.)	8097	13.8	9259	15.2	11928	17.6
Other neoplasms	178	0.3	201	0.3	253	0.4
Diabetes mellitus	1294	2.2	1656	2.7	2229	3.3
Nutritional/endocrine disorders	310	0.5	331	0.5	395	0.6
Neuropsychiatric disorders (like schizophrenia, epilepsy, alcohol use disorders, Alzheimer and other dementias, Parkinson disease, drug use disorder etc.)	1320	2.2	1429	2.3	1757	2.6
Sense organ disorders	5	0.0	5	0.0	6	0.0
Cardiovascular diseases	17890	30.4	19388	31.9	23578	34.8
Respiratory diseases (like chronic obstructive pulmonary disease, asthma)	4426	7.5	5220	8.6	7373	10.9
Digestive diseases (like peptic ulcer disease, cirrhosis of the liver etc.)	2010	3.4	2015	3.3	2164	3.2
Diseases of the genitourinary system (like Nephritis/nephrosis, benign prostrate hypertrophy)	980	1.7	1089	1.8	1376	2.0
Skin diseases	71	0.1	80	0.1	103	0.2
Musculoskeletal diseases	131	0.2	142	0.2	175	0.3
Congenital abnormalities	408	0.7	373	0.6	294	0.4
Oral diseases	3	0.0	4	0.0	5	0.0
III. Injuries	**5663**	**9.6**	**5957**	**9.8**	**6801**	**10.0**
Unintentional injuries	3977	6.8	4181	6.9	4786	7.1
Intentional injuries	1685	2.9	1777	2.9	2015	3.0

Table 3: Projected deaths by cause, globally [72].

The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportions of people >65 years of age. The three nations which top the list in prevalence of diabetes are India, China and U.S. with estimated 31.7, 20.8 and 17.7 million people affected by diabetes in the year 2000; is projected to reach 79.4, 42.3 and 30.3 million people in the year 2030, respectively.

Cardio-vascular diseases

As the average human life expectancy has increased, so has the impact of ageing and age-related diseases like CVDs. CVDs are a group of disorders [75] of the heart and blood vessels and include (a) coronary heart disease – disease of the blood vessels supplying the heart muscle, (b) cerebrovascular disease - disease of the blood vessels supplying the brain, (c) peripheral arterial disease – disease of blood vessels supplying the arms and legs, (d) rheumatic heart disease – damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria, (e) congenital heart disease - malformations of heart structure existing at birth and (f) deep vein thrombosis and pulmonary embolism – blood clots in the leg veins, which can dislodge and move to the heart and lungs.

One of the major causes of CVDs [76] is essential hypertension, known as the silent killer, which does not cause symptoms for many years until a vital organ is damaged [77]. Essential hypertension is multi-factorial in origin [78], cannot be cured but can be controlled [79-80] effectively by use of medicine [81]. The other non-pharmacological approach [82] could be through (a) weight control [83-85], (b) sodium restriction [86-90], (c) fat content [90], (d) alcohol restriction [91-92], (e) physical exercise [93-94], (f) relaxation therapies for stress reduction [95-97] and (g) potassium therapy [98-99].

CVDs are the number one cause of death globally [100]. An estimated 17.1 million people died from CVDs (29% of all global deaths) in 2004. Of these deaths, ~7.2 million and ~5.7 million deaths were due to coronary heart disease and stroke, respectively. 82% of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women. It is projected that by 2030, almost 23.6 million people will die from CVDs, mainly from heart disease and stroke, with largest increase in number of deaths would be in the South-East Asia Region, WHO [101]. There are currently about 800

million people with high BP worldwide. The current prevalence in many developing countries, particularly in urban societies, is already as high as those seen in developed countries [102-103]. Studies indicate that (i) by lowering of each 10 mmHg of systolic BP, there is 1/3rd decrease in risk of stroke in people of age between 60-79 years; (ii) by lowering diastolic blood pressure (DBP) by 2-7% below 95 mmHg, million deaths per year from coronary heart disease and stroke can averted by 2020.

Cancer

Cancer [104] is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. It arises from one single cell. The transformation from a normal cell into a tumour cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumours. These changes are the result of the interaction between a person's genetic factors and three categories of external agents, including (i) physical carcinogens, such as ultraviolet and ionizing radiation, (ii) chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant) and arsenic (a drinking water contaminant) and (iii) biological carcinogens, such as infections from certain viruses, bacteria or parasites. The relative importance of the most common cancers [72] in terms of number of deaths at all ages is summarized in Table 4.

Globally lung cancer (including trachea and bronchus cancers) are the most common cause of death from cancer among men, and stomach cancer mortality is second. Colon and rectum cancers are the 4th leading cause & oesophagus cancer the 5th leading cause globally. Prostate cancer is the 6th globally. For woman, 15 cancers are ranked, of which the most common is the breast cancer, followed by cancers of trachea, bronchus, lung and stomach cancer. Other cancers of female reproductive system are Cervix uteri (5th), ovary (8th) and Corpus uteri (13th) the leading cause of death in woman.

In 2005, 7.6 million people died of cancer [105]. WHO [106] projects that with steadily increasing proportion of elderly people in the world will result in approximately 50% increase in new cancer cases over next 20 years. From the studies based on 5-year prevalence between 1998-2002, WHO projects that the number of people affecting from cancer would rise from ~10.9 million in 2002 to ~16 million in 2020, almost nearly a 50% increase; 2/3rd of them would be from newly industrialized and developing countries. It is estimated that almost 7 million people would die each year of cancer and 10.3 million by 2020 unless proper measures are not taken. Dr. John R. Seffrin, President, UICC states, "Cancer is potentially the most preventable and most curable of the major life-threatening disease facing human kind. We can save 2 million lives by 2020 and 6.5 million by 2040."

S. No.	Type of Cancer	Ranking in Men	Ranking in Women
1.	Trachea, brochus, lung cancers	1	2
2.	Stomach cancer	2	3
3.	Liver cancer	3	6
4.	Colon and rectum cancers	4	4
5.	Oesophagus cancer	5	7
6.	Prostrate cancer	6	-
7.	Mouth and oropharynx cancers	7	12
8.	Lymphomas & multiple myeloma	8	9
9.	Leukaemia	9	11
10.	Bladder cancer	10	14
11.	Pancreas cancer	11	10
12.	Melanoma & other skin cancers	12	15
13.	Breast cancer	-	1
14.	Cervix uteri cancer	-	5
15.	Ovary cancer	-	8
16.	Corpus uteri cancer	-	13

Table 4: Ranking of most common cancer among men and women according to number of deaths, by cancer site, WHO worldwide data, 2004 [72].

Chronic respiratory disease

As per WHO [107] "chronic respiratory diseases" are chronic diseases of the airways and other structures of the lung. Some of the most common are asthma, chronic obstructive pulmonary disease (COPD), respiratory allergies, occupational lung diseases and pulmonary hypertension.

Asthma [108] is a chronic disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. Symptoms may occur several times in a day or week in affected individuals, and for some people become worse during physical activity or at night. Asthma is the most common chronic disease amongst children. According to WHO estimates, 300 million people suffer from asthma and 0.25 million people died of asthma in 2005. COPD [109] is a lung ailment that is characterized by a persistent blockage of airflow from the lungs. It is an under-diagnosed, life-threatening lung disease that interferes with normal breathing and is not fully reversible. An estimated 210 million people have COPD worldwide. More than 3 million people died of COPD in 2005, which is equal to 5% of all deaths globally that year. The primary cause of COPD is tobacco smoke (through tobacco use or second-hand smoke). Total deaths from COPD are projected to increase by more than 30% in the next 10 years without interventions to cut risks, particularly exposure to tobacco smoke. Allergic rhinitis or hay fever [110] happens when one breathes in something to which he/she is allergic, and the inside of the nose becomes inflamed and swollen. Sinusitis [110] is an inflammation of the lining inside the sinuses which can be acute or chronic. When the sinuses become blocked and fill with fluid, germs can grow and cause symptoms such as headache and nasal yellowish secretions. Blocked sinuses can be caused by the common cold, hay fever or nasal polyps (small lumps inside the nose). Allergic rhinitis and sinusitis are linked to each other. Acute sinusitis usually subsides without any need for specific treatment. Chronic sinusitis may require antibiotics, decongestants or steroid nasal sprays. Pulmonary hypertension [111] is a condition in which there is high blood pressure in the lung arteries as the arteries become narrow affecting the blood flow. Over time, some of the arteries may stiffen and become completely blocked, causing the right side of heart to work harder to pump blood through the lungs. Over time, the heart muscle weakens and loses its ability to pump enough blood for the body's needs. The extra stress causes the heart to enlarge and become less flexible. Heart failure is one of the most common causes of death in people who have pulmonary hypertension. In some cases, pulmonary hypertension is caused by *schistosomiasis*, a worm infection common in Africa and Latin America; and sickle cell disease, a genetic abnormality of blood which is common in persons of African origin. Difficulty in breathing or shortness of breath is the main symptom of pulmonary hypertension. Other symptoms are fatigue, dizziness, swelling in the ankles or legs (edema), bluish lips and

skin (cyanosis), chest pain, racing pulse and palpitations. There are no mortality details on the allergic rhinitis or hay fever and pulmonary hypertension on WHO website.

Pharmacognostic Aspects

Common name

Artemisia afra is known by many names like "African wormwood" in English "Umhlonyane" in Xhosa, "Mhlonyane" in Zulu, "Lanyana" in Sotho, "Lengana" in Tswana, "Wilde als" in Africaans, "Koddoo-adi" & "Chugughee" in Ethiopia [29,34,112,113]. It is also known by other names viz., Als, Wild wormwood, Fivi, Lusanje, Luyanga, Iliongana [114].

The genus name *Artemisia* is derived in honor of the Greek goddess of hunting Artemis [115]. Another story [116] goes that the name is kept after Artemisia, the famous botanical and medical researcher and the wife of the Greek/Persian King Mausolus, who built a magnificent Mausoleum tomb known as seven Wonders of the Ancient World, after his death in 353 BC.

Taxonomy

Artemisia afra belongs to Domain: *Eukaryota*, Kingdom: *Plantae*, Subkingdom: *Viridaeplantae*, Phylum: *Tracheophyta*, Subphylum: *Euphyllphytina*, Infraphylum: *Radiatopses*, Class: *Magnoiopsida*, Subclass: *Asteridae*, Superorder: *Asteranae*, Order: *Asterales*, Family: *Asteraceae*, Subfamily: *Asteroideae*, Tribe: *Anthemideae*, Genus: *Artemisia*, Specific ephithet: *afra*- Jacq., Botanical name: *Artemisia afra* [117]. The *Asteraceae* is one of the most important family of plants in the world. More than 23000 species from about 1300 genera have been identified [118]. Many species have been used as sources of rubber, medicines, edible oils, vegetables, pesticides and so on. Some are popular ornamental plants. The genus *Artemisia* contains more than 400 species [119,120].

Phylogeny

The review paper by Hayat et al. [121] discusses the development in the classification and phylogeny of genus *Artemisia* L. They report that this plant group could have been originated in temperate Asia (mesothermic subarctic or semihumid environments prevailing near Ural Mountains); in the mid-tertiary period of Cenozoic era, and the centers of diversity of this genus could be in the temperate and cold temperate regions of Eurasia, North America and Asia. The paper discusses the basis of disagreements amongst the Scientists with respect to taxonomic treatment of *Artemisia* in the last half decade for maintaining a single large genus of over 500 species to the recognition of six to eight genera within its taxonomic boundaries. The conclusion of (i) palynological, (ii) karyological, (iii) floral & capitular morphological and (iv) molecular phylogenetic studies for pollen evaluation, chromosomal counts & polyploidy, evolution of floral characters and molecular phylogeny respectively undertaken by various researchers are reported.

Geographical distribution

A. afra is a herb growing in the high land areas of Eastern and Southern Africa altitudes ranging between 1500 and 3000m where the soils range from volcanic ash, loamy sands, to sandy or calcareous clay loams of volcanic or granitic origin [114,122]. The plant grows in the South and Eastern regions of the continent and has been located in Ethiopia, Kenya, Tanzania, Zaire, Zambia, Zimbabwe, Angola and the Republic of South Africa [52,122]. In South Africa, it usually grows in rocky mountainous areas along forest margins and stream sides and its natural distribution extends from the Northern and Eastern Transvaal to the Western Cape, except the Northern Cape [123]. It is also predominantly found in Asia, Europe and North America [119,120]. The geographical distribution of *A. afra* in South Africa [124] with the copyright permission from Scott & Springfield [125] is given in Figure 4. It's one of the domesticated plant in these regions [126].

Plant description

Artemisia afra is a medium sized multi-stemmed, clump-forming woody perennial shrub, which grows up to 2 meters in height with a leafy, hairy ridged stem [6,112].

Its soft leaves are finely-divided (like a fern), are silver-grey due to the presence of fine hairs reaching in length up to 80 mm and

Figure 4: Distribution of *A. afra* in South Africa [Scott, G. and Springfield, E. P. (2004). Artemisia Afra Herba. In: Pharmaceutical Monographs on CDROM for 60 South African plant species used as traditional medicines. South African National Biodiversity Institute, Pretoria [125]. Reproduced with copyright permission from authors.

Figure 5: African wormwood *Artemisia afra* plant (a) growing branch from bbc.co.uk/gardening [128] and (b) fully grown plant from plantzafrica.com [112].

Figure 6: The flowering stem containing yellow florets and buds from Fabian & Germishuizen, 1997 [130].

width up to 40 mm arranged alternately, oval in shape [11,53,127]. An image from BBC Magazines Ltd. [128] is given in Figure 5a. The adaxial surface of the leaf is darker compared to its abaxial surface [6]. Figure 5b is the picture of fully grown plant from the South African Biodiversity Institute's Plant Information [112]. The plant has an easily identifiable aromatic odour and smells pungent and sweet after bruising [129]. It produces pale yellow tubular florets, with few outer female and inner bisexual florets occurring in an elongated racemose panicle, an image from the book, Wild flowers of Northen South Africa [130] is given, Figure 6.

The capitula are small, receptacle flat and naked. The African wormwood produces small, inconspicuous wild fertilized flowers between March and July, and the seeds are produced from August to November. The fruits are about 1 mm long, somewhat 3-angled and slightly curved with a silvery-white coating [11,53,56,131].

Histology

The salient microscopic properties presented here are obtained from the Monograph of "*Artemisia afra* Herba" given by South African Medical Council Research, South Africa Health Information [124] and copyright permission [125], Figure 7 (i –ix). (i) Fibrous layer of anther, (ii) corolla showing papillate inner epidermis, (iii) tricolporate yellow-brown pollen grains, ± 20 μ in diameter, (iv) polygonal epidermal cells of upper leaf lamina, (v) small block-like cells of stamen filament, (vi) epidermal cells of lower leaf lamina with sinuous slightly thickened walls, (vii) fragment of corolla (tubular floret) with microcrystals of calcium oxalate, (viii) vessels of stamen filament and (ix) fragments of corolla with striated outer epidermis.

Cultivation and collection

The intensive harvesting of medicinal plants for use and commercial trade in South Africa poses a threat to many species. Hence, cultivation has been considered as an alternative to collection in the wild. Keirungi and Fabricius [118] assessed the feasibility of cultivating 17 selected medicinal plants based on its medicinal importance in Nqabara Administrative Area on South Africa's Wild Coast and reported that *A. afra* holds 8th rank in the list of important medicinal plant with a market value.

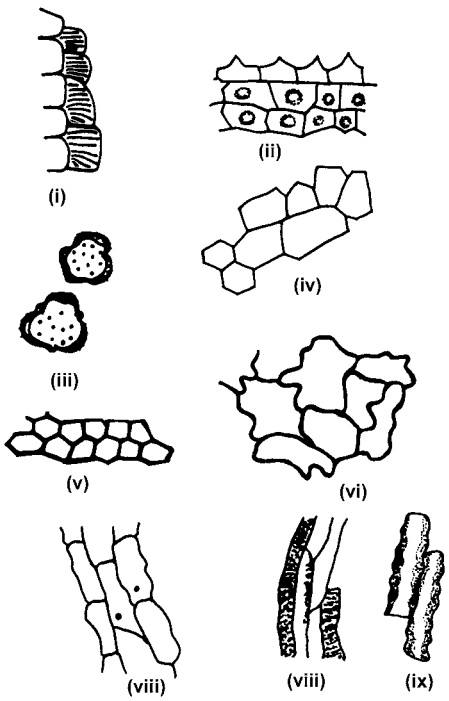

Figure 7: Salient identifying microscopic characters of *A. afra*. Scott, G. and Springfield, E. P. (2004). Artemisia Afra Herba. In: Pharmaceutical Monographs on CD-ROM for 60 South African plant species used as traditional medicines. South African National Biodiversity Institute, Pretoria [125]. Reproduced with copyright permission from authors.

Factors affecting cultivation of medicinal plants: The main factors which generally affect the cultivation of medicinal plants can be stated as (i) proximity to plant source, (ii) time spent in collecting the plant, (iii) number of ailments perceived to be healed i.e., frequency of usage, (iv) retention of activity, (v) acceptance to use plants which are cultivated, (vi) ease to cultivate - availability of seeds or grafts, soil quality, venerability to pests, modest water requirements, maintenance etc., (vii) impact on other plants, (viii) market value and economic potential and (ix) awareness amongst people that they have been causing adverse effect on large trees due to plucking shrubs around its vicinity and willingness to conserve indigenous forests.

Habitat: *A. afra* is very drought resistant and hardy [53], common to arid soils [131], open to sunny situation with light, well drained soil [131], needing water occasionally [53]. The optimal temperature and annual rain fall as described in FAO *Artemisia afra* Data Sheet are 22-33°C and 550-750 mm respectively [114].

Cultivation: The Plant Biology Guide to Growing Artemisia Wormwood [132] mentions that the seeds can be sown either in spring or autumn, in well drained soil at a pH of 5.5 to 7. The soil should be kept moist until germination takes places, which is normally 2-8 weeks. The seedlings should be put out after the last frost and planted from 30 cm (small species) to 60 cm (larger species). Fertilizer should be applied in the early spring, and mulch applied in the late autumn. The leaves can be harvested any time. A very quick and easy way to propagate is from cuttings [53] in summer and by division in spring, prune in spring to stimulate growth. These would grow in any soil and needs just occasional watering and cutting back.

The South Africa Department of Agriculture, Directorate of Plant Production, Division of Industrial Crops [133] has compiled in collaboration with members of South African Essential Oils Producer Association (SAEOPA) and KARWIL consultancy all the vital aspects involved in the production of *Artemisia afra*. It is made freely available on the official website of Directorate of Agriculture Information Services viz., http://www.nda.agric.za/publications. It is a 26 page document comprising 5 parts along with references for further reading. It details general aspects (like classification, origin & distribution, production levels, major production areas in South Africa, plant description, climatic & soil requirements); cultivation practices (like propagation, soil preparation & planting, fertilization & irrigation; pest, disease & weed control, mulching & harvesting); post-harvest handling (like sorting & distillation, grading; packaging, storage & marketing); production schedule and finally its utilization.

Choice of genotypes: Graven et al. [134] demonstrated that by selecting superior genotypes from the wild and cultivating it, provides major opportunities for the economic advancement of the new crop as the chemical composition in a plant is genetically determined and should not affect when cultivated. The thujone content in wild *A. afra* plants varies from 10% to 93% between individual plants. When the mother plant containing 91% thujone was selected and propagated by root cuttings, the results showed that the thujone content of the vegetatively propagated cuttings did not vary by more than 2% over 5 generations of vegetative propagation. The author suggests that the identification of mother plants having the desired chemical composition, coupled with vegetative propagation will retain the desired genetic characteristics, and facilitate the development of superior clones for cultivation. In this manner, clones can be developed which exceed the minimum standards for the active ingredients as required by the phyto-medicinal and essential oil industries.

Socio-economic impact: A study by team of Wiersum et al. [135] was carried out in the Amatola region of Eastern Cape, South Africa,

to assess whether cultivation of medicinal plants can serve as a tool for combined biodiversity conservation and poverty alleviation. The natives were found to use more than 100 plants, of which over 50 species were found to be cultivated in the home gardens. *A. afra* topped in the cultivation frequency list standing at 40%. The authors conclude that cultivation of medicinal plants play a significant role in the maintenance of cultural identity, increased human capital and dignity by alleviating poverty. However, one should not be too optimistic about the scope of medicinal plant cultivation by poor people as a practical strategy for *ín domo* conservation of threatened species as (i) the preferred species for cultivation may not necessarily be the most threatened species and (ii) it was still not clear whether such cultivation substitutes the collection of wild species or supplements it.

Chemical Constituents

There are extensive data showing that the flavonoid synthesis is influenced by different abiotic (geographical variation, UV light radiation, drought, ozone), biotic (phytopathogens, insect detterents) factors [136]. Also, there are human factors like method of cultivation, processing parameters (collection, drying etc.) and extraction techniques that influence the plant constituents both qualitatively and quantitatively. These are discussed in this section.

Method of extraction on yield and chemical composition

Asfaw et al. [137] studied the four different methods of extraction viz., (i) hydrodistillation (HD), (ii) microwave assisted extraction (MAE), (iii) ultrasound assisted extraction (UAE), and (iv) liquid/supercritical CO_2 extraction (l-CO_2 and sc-CO_2) to determine its extractive property both in terms of yield and chemical composition from *A. afra* plant. In general, the essential oil obtained from each method of extraction were similar in appearance - pale colored and fragranced. The details of the components with Kováts indices determined by Gas Chromatography – Flame Ionization Detector (GC-FID) from 900 to 1350 are given in Table 5. The yields were highest with I-CO_2 and sc-CO_2 (3.2% v/w), followed by traditional HD (1.5% v/w). The lowest yield was obtained with UAE (0.7% v/w). When a comparison of different fractions obtained was made for yogomi alcohol content in the extracts obtained from I-CO_2 and sc-CO_2, UAE, MAE and HD, it was found to be 0.4%, 0.4%, 0.1%, 3.6% and 8.1% respectively. Eight sesquiterpenes could only be detected in the sc-CO_2, I-CO_2 and UAE, with relative percentage peak areas of 13%, 16% and 29%, respectively. The differences could be ascribed to the solubility differences or instability of compounds during the different methods of extraction.

Method of analysis

To identify the major components in the oil, the only reported analytical equipment used was gas chromatography coupled with either a flame ionization detector or mass spectroscopy detector [137]. Liu et al. [129] classified and compiled 131 volatile secondary metabolites and 44 non-volatile secondary metabolites from *A. afra* oil in 4 and 10 categories from 16 and 8 published papers respectively to date and is summarized in the Table 6.

Geographical variation

The main components of the volatile secondary metabolites in *A. afra* varied enormously in plants from different geographical regions. The major constituent in Ethiopian oil [138] was artemisyl acetate (24.4–32.1%) while it was 1,8-cineole (67.4%) in Kenyan oil [139]. In Zimbabwean oil, α- and β-thujone (52%) was the major constituent [140] while α-thujone (54.2%) was in South African oil [141].

Viljoen et al. [142] analyzed the hydro-distilled essential oil by GC-MS obtained from aerial parts of 16 individual *A. afra* plants collected from four natural population [viz., 3 plants each from Setibeng (Lesotho), Giant's Castle (KwaZulu-Natal), Qwa-qwa (Free State) and 7 plants from Klipriversberg (Gauteng)] and found that quantitative and qualitative variation within and between natural populations with no correlation to the geographical distribution.

Oyedeji et al. [43] studied the α-thujone content isolated from

I_k[a]	Compound	Relative peak areas (%)				
		$scCO_2$[b]	I-CO_2[c]	Sonic[d]	μ-wave[e]	HD[f]
903	Santonlina triene	0.6	0.6	1.7	2.3	2.1
917	A-Pinene	-	-	-	0.4	0.8
956	Camphene	0.6	0.6	0.5	0.9	0.6
999	Yogomi alcohol	0.4	0.4	0.1	3.6	8.1
1027	Limonene	2.6	4.1	2.5	4.8	3.6
1033	1,8-Cineole	2.2	1.4	1.6	3.0	2.9
1061	Artemisia ketone	6.8	9.9	7.1	13.3	12.4
1080	Linalool	0.3	0.3	0.2	0.8	1.7
1116	*p*-Menthatriene	2.4	2.5	1.8	1.4	1.0
1172	Artemisia acetate	22.4	17.4	12.7	25.6	26.8
1174	Aretmisia alcohol	11.3	14.5	11.4	14.7	9.9
1255	Geraniol	4.5	5.5	2.9	6.2	6.2
1305	Bornyl acetate	3.6	2.2	2.5	4.1	8.2
	8 Sesquiterpenes	13	16	29	-	-

[a]Experimentally determined Kováts indices on the DB-5 column.
[b]$scCO_2$: Extraction carried out for 20 min. at 50 °C, 100 bar, liquid CO_2 (at -10 °C) was delivered to the extractor vessel at a constant flow rate of 5 mL min^{-1}.
[c]I-CO_2: Extraction carried out for 20 min. at 30 °C, 100 bar, liquid CO_2 (at -10 °C) was delivered to the extractor vessel at a constant flow rate of 5 mL min^{-1}.
[d]Ultrasonic irradiation for 30 min., in diethyl ether at ambient temperature and pressure.
[e]Microwave irradiation for 10 min, at ambient pressure.
[f]Hydrodistilation for 180 min.
Green Chemistry, 7, N. Asfaw, P. Licence, A. A. Novitskii, M. Poliakoff, Green chemistry in Ethiopia: The cleaner extraction of essential oils from Artemisia afra: a comparison of clean technology with conventional methodology, 352-356,2005]. Reproduced by permission of The Royal Society of Chemistry [137].

Table 5: A. afra components determined by GC-FID analysis.

S. No.	Component Type	No. of Metabolites
	A. Volatile Secondary Metabolites	
1.	Monoterpenoids	83
2.	Sesquiterpenes	30
3.	Others	15
4.	Probably contained compounds	3
	Total	**131**
	B. Non-volatile Secondary Metabolites	
5.	Sesquiterpenes	1
6.	Glaucolides	7
7.	Guaianolides	6
8.	Others	1
9.	Triterpenes	4
10.	Long chain alkanes	6
11.	Coumarins	5
12.	Organic acids	1
13.	Glycosides	1
14.	Flavonoids	11
	Total	**43**

Table 6: Volatile and non-volatile secondary metabolites in *A. afra*.

essential oil obtained by hydrodistillation of twigs of *A. afra* plants obtained from different locations in the Eastern Cape, Free State and KwaZulu-Natal by GC and GCMS. Their analysis revealed compositional variations in the levels of α- and β-thujone, 1,8-cineole and camphor. α-thujone was the major component of the essential oils of *A. afra* from Philippolis (Free State) and Keiskammahoek (Eastern Cape) (62-74%), while the camphor content was very low (≤0.1-0.6%). The samples from Gqumahshe, Hogsback (Eastern Cape) and Empangeni (KwaZulu Natal) had low α-thujone contents (3.7-20.0%) while 1,8-cineole (13.0-49.5%) and camphor (13.9-21.2%) were the main components of the essential oils.

Avula et al. [44] estimated five flavonoids viz., (i) apigenin, (ii) chrysoeriol, (iii) tamarixetin, (iv) acacetin and (v) and genkwanin (Figure 8) by HPLC-UV and HPLC-MS technique to determine flavonoids in the aerial parts of the 11 samples of *Artemisia afra* Jacq. ex Willd plant obtained from widely separated populations in the provinces of Kwa-Zulu Natal and the Western Cape in South Africa. Figure 9 was plotted from the data published by them, which shows that the geographical variation does effect the total % content of flavonoids and the proportions of which varies in almost all samples accordingly for each ingredient, seen by uncrossed curves in the graph. Other details are given in under the heading "Analysis and Quality Control".

Effect of cultivation

Chagonda et al. [143] reports the difference in volatile oils obtained from wild and organically cultivated plants of *A. afra* (Jacq.) (Compositae) from two pilot sites in Zimbabwe. The oil yield from the cultivated plant was between 0.33 and 0.60% (v/w), greenish- to brownish-yellow in color. Oils from the two cultivated sites had pleasant but different and distinct odours. The constituents of volatile secondary metabolites obtained by steam distillation were analyzed by GC-MS and are given in Table 7. Analysis of oils from fresh cultivated *A. afra* showed the presence of two chemotypes: one dominated by artemisia ketones (32.1-34.8%), α-copaene/camphor (21.8-24.4%) and 1,8-cineole (10.9-16.9%) and cultivated in Harare, and the other by 1,8 cineole (23.5-28.7%), α-copaene/camphor (20.2-21.3%) and borneol (14.2-17.0%) cultivated in Murehwa. The cineole chemotype had, as other notable minor components, bornyl acetate (1.6-3.3%), β-caryophylene (2.0-5.0%), sabinene (0.6-7.9%) and camphene (3.0-

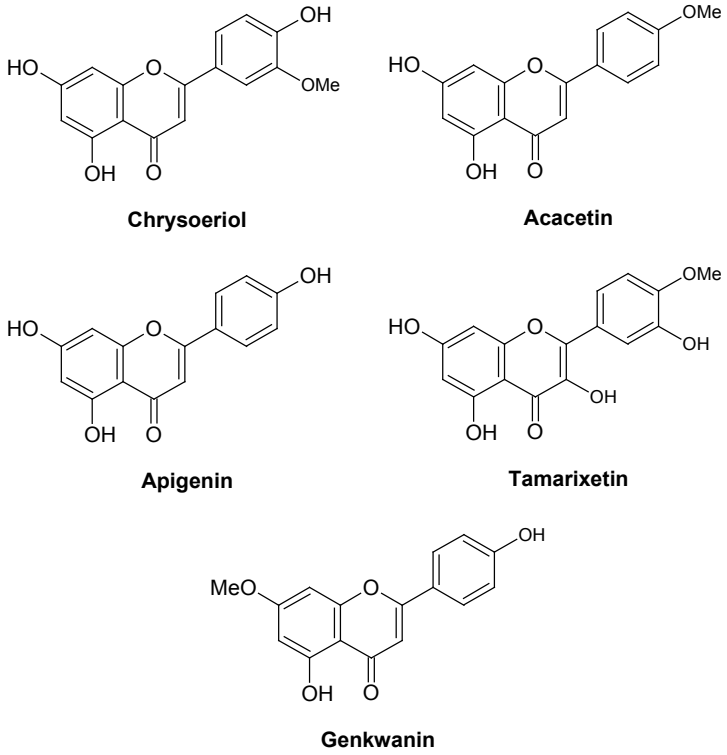

Figure 8: Structure of flavonoids from [44].

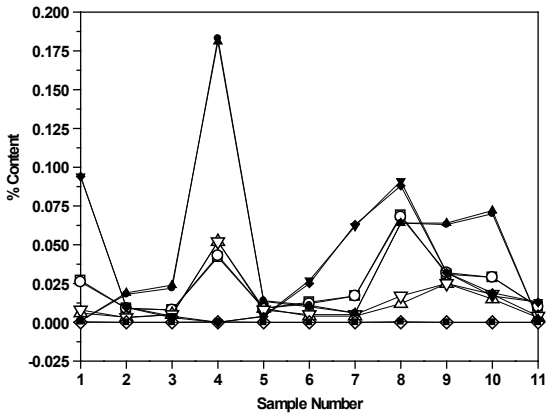

Figure 9: % Content of flavonoids of Apigenin (□), Chrysoeriol (O), Tamarixetin (Δ) Acacetin ( ) and Genkwanin (◊) by by HPLC–UV method and Apigenin (■), Chrysoeriol (●), Tamarixetin (▲) Acacetin (▼) and Genkwanin () by HPLC-MS method.

5.6%). The oil from the semi-dried plant material with the cineole chemotype had a similar pattern in its oil composition to that from the fresh plant and contained 1,8-cineole (22.5-29.3%), borneol (17.9-19.1%) and α-copaene/camphor (6.2-19.9%) as the major components. Bornyl acetate (2.7-4.2%), β-caryophyllene (2.0-2.8%) and camphene (3.4-3.5%) were notable minor constituents. Differences in oil composition were observed between fresh and semi-dried plant material and dry plant material (winter post-harvested collected dry plant B_o with the later yielding α-copaene/camphor (50.6%) as the major component.

Method of drying

The impact of drying methods on the quantity and quality of the essential oil of *A. afra* was studied by Asekun et al. [144]. The yields of oil from the plant differed according to the drying methods; viz: 0.18%, 0.88%, 1.54% and 1.88% for fresh, oven-dried, air-dried and sun-dried oils, respectively. They also found that the oil extracted from fresh plants contained artemisia ketone (6.9%) which was absent in the oil extracts obtained from air- and sun dried plants. Extracts from sun-dried plants had 14 components and the lowest number. Oyedeji et al [43], studied the α-thujone content isolated from essential oil obtained by hydrodistillation of fresh and dried twigs of *A. afra* plants by GC and GC-MS and found that the concentration of α-thujone increased significantly in the dry leaves when compared with the fresh leaves.

Variation between plants

It is already dealt under the heading "Geographical variation", that

No.	Component†	A = wild	B = cultivated UZ Farm				C = cultivated Murehwa			
		A (%) 1996 *n* = (3)	B_o (%) 9/1996 (1)	B_1 (%) 5/97 (3)	B_2 (%) 8/97 (1)	B_3 (%) 4/98 (2)	C_1 (%) 5/97 (1)	C_2 (%) 3/98 (1)	C_3 (%) 4/98 (1)	S/dry herb C_4 (%) (2)
1	Tricylene	0.1-0.2	tr	0.1-0.2	0.1	0.0-0.1	0.1	0.3	---	tr-0.2
2 + 3	α-Pinene + α-thujone	0.4-1.1	0.3	0.5	0.8	0.5-1.1	0.7	1.3	1.75	0.9-1.1
4	α-Fenchene	0.2-1.0	1.4	0.6-1.1	0.7	0.4-0.9	---	0.6	---	tr
5	Camphene	0.3-3.9	8.0	3.9-4.0	0.6	3.0-3.8	3.0	5.2	5.6	3.4-4.5
6	β-Pinene	0.1-0.7	0.4	0.3-0.4	3.7	0.2-0.3	0.3	1.5	0.5	0.3-0.5
7	Sabinene	0.1-2.6	0.2	0.3-0.4	0.3	0.2-0.5	7.9	0.6	7.3	1.5-6.3
8	Myrcene	0.1-1.0	1.6	0.5	0.3		0.6	1.0	1.1	tr-0.6
9	α-Terpinene	0.1-1.1	0.3	0.3	---	0.2-1.8	0.5	0.3	1.1	0.6-1.3
10	Dehydro-1,8-cineole	0.1-0.2	0.1	0.1	0.7	tr-0.3	0.2	0.7	0.2	tr-0.2
11	Limonene	0.1-0.5	0.9	0.2	0.1	0.1	0.5			tr-0.4
12	**1,8-Cineole**	0.1-27.9	10.7	16.5-16-9	15.7	10.9-15.3	**23.5**	**25.1**	**28.7**	**22.5-29.3**
13	(*E*)-β-Ocimene	0.1-0.3	tr	0.2	---	tr-1.0	---	---	---	tr-0.4
14	γ-Terpinolene	0.3-1.9	0.7	0.6	---	tr-0.2	1.3	0.2	2.6	1.3-2.4
15	*p*-Cymene	0.3-2.0	0.8	1.0-1.1	1.6	0.6-1.5	1.2	1.3	1.2	2.2-2.7
16	Terpinolene	0.1-0.5	0.3	0.2	---	tr-0.2	0.3	0.5	0.5	0.3-0.6
17	**Artemisia ketone**	**6.3-41.9**	**0.1**	**32.1-32.5**	**34-8**	**32.1-33.1**	0.1	0.3	0.1	tr-0.4
18	**Santolina alcohol**	**3.1-10.1**	---	**2.5-4.5**	**8.0**	**2.7-4.3**	0.1	0.1	0.1	tr-0.1
19	α-Thujone	1.0-2.9	0.7	0.5	0.5	0.2-4.9	0.1	0.1	0.1	tr
20	Artemisyl acetate	tr-0.1	---	---	0.5	1.0-0.9	0.1	---	0.2	tr-0.1
21	β-Thujone	tr	---	---	---	tr-0.2	---	tr	tr	tr-0.3
22	*cis*-Sabinene hydrate	0.2-0.5	0.4	0.1	---	tr-0.2	1.3	0.8	1.3	1.0
23	Artemisia alcohol	tr-0.3	---	0.1	---	tr-0.1	---	0.1	---	tr-0.2
24/25	**α-Copaene/Camphor**	**8.5-27.1**	**50.3**	**23.0-23.1**	**21.8**	**24.3-24.4**	**20.6**	**21.3**	**20.2**	**6.2-19.9**
26	*trans*-Sabinene hydrate	1.8-4.4	3.5	0.1-3.5	3.5	3.0-3.7	---	---	---	tr-0.1
27	*cis-p*-Menth-2-en-1-ol	0.2-0.4	0.8	0.1-0.2	---	0.1-0.5	1.0	0.9	1.5	1.0
28	Bornyl acetate	0.3-1.5	0.5	0.2-0.3	0.7	0.6-0.7	3.3	1.6	1.8	2.7-4.2
29	β-Caryophyllene	0.5-2.3	1.2	0.7	0.7	0.4-0.8	5.0	2.0	2.4	2.0-2.8
30	Terpinene-4-ol	tr-0.1	---	0.1	---	tr-0.1	0.5	0.3	0.7	tr-0.8
31	Myrtenal	---	---	0.1	---	---	0.6	0.4	0.3	tr
32	*trans-p*-Meth-2-en-1-ol	0.2-0.3	---	0.1	---	0.1-0.2	0.4	0.3	0.2	tr-0.3
33	Not identified	tr-0.1	---	0.1	---	0.2	0.1	0.2	0.1	tr-1.3

34	δ-Terpineol	0.1-2.5	0.7	0.3	---	tr-0.5	1.0	0.9	0.9	tr-0.1
35	**Borneol**	0.6-3.4	2.8	0.8-0.9	0.8	1.4-	**17.0**	**15.3**	**14.2**	**17.9-19.1**
36	**α-Terpineol**	0.1-0.7	0.4	0.2-0.3	0.4	2.4	**1.9**	**0.9**	**0.2**	**0.2-8.1**
37	Bicyclogermacrene	0.2-0.5	---	0.3	---	0.5-0.8	0.2	---	0.2	---
38	Piperitol*	0.1-0.7	1.8	0.4	---	tr-0.6	0.1	0.1	---	tr-0.1
39	δ-Cadinene	0.5-0.8	0.2	0.6	0.6	1.0-1.2	1.9	1.7	1.6	0.9-1.0
40	Cuminaldehyde	tr-0.5	0.2	0.1	---	0.1-0.7	0.2	tr	0.2	tr-0.3
41	Myrtenol	tr-0.1	0.3	0.1	---	0.1-0.1	0.2	0.2	0.2	tr-0.1
42	Calamenene*	0.1-0.9	0.1	0.1	---	0.1	0.4	0.1	0.2	tr-0.2
43	*cis*-Carveol	---	0.1	---	---	0.1	0.1	tr	0.1	tr
44	Not identified	---	0.1	tr	---	---	0.1	tr	0.1	tr-0.1
45	Not identified	---	0.3	tr	---	---	0.1	0.1	0.1	tr
46	*trans*-Caryophyllene oxide	tr-0.1	0.1	0.1	---	0.1	0.1	0.1	0.2	tr-0.1
47	Methyl linolenate	tr-0.1	---	---	---	---	0.1	---	0.1	tr-0.1
48	Germacrene-D-4-ol	---	0.1	---	---	---	0.1	---	---	tr
49	Methyl linolenate**	---	0.1	---	---	---	0.1	---	0.2	tr
50	*p*-Cymen-8-ol	---	0.1	tr	---	---	0.1	0.1	---	tr-0.1
51	Spathulenol	---	---	---	---	0.3-0.6	0.1	0.2	---	---
52	T-muurolol	tr-0.5	0.4	0.1	---	tr-0.6	0.2	---	0.1	0.4-0.6
53	Intermedeol	tr-0.4	0.1	---	0.3	0.1-0.2	0.2	0.1	0.1	tr
54	Not identified	---	---	---	---	---	0.4	---	---	tr-1.7
† Identified by GC-MS; * Isomer not identified; ** Tentative. *n* = no. of batches.										
Flavor and Fragrance Journal, 14 (2), Chagonda L. S, Chalchat, J-Claude, The essential oil of cultivated Artemisia afra (Jacq.) from Zmbabwe, 140-142, 1999. Reproduced by permission of John Wiley and Sons [143].										

Table 7: Percentage of major constituents in wild and cultivated *A. afra*.

Substance	IC_{50} (µgm/ml)		Selectivity Index (SI)
	T. b. brucei	HL-60	
MeOH Extract	77.54	132.97	1.71
CH_2Cl_2 Extract	25.27	123.21	4.87
Diminazene aceturate drug	0.088	>128.88	>1464.00

Table 8: Trypanocidal and cytotoxic activities of artemisinin and crude extract from *A. afra*.

the constituent do vary between two plants.

Variation within plants

The oil obtained from different parts of the same plant showed variation in the constituents [142]. Goodson [24] found camphor, a wax ester, triacontane, scopoletin and quebrachitol in the flowering tops of *A. afra*. Bohlmann and Zdero [25] revealed that the roots of *A. afra* contained isomeric courmarins and five acetylenes, while the aerial parts contained thujone and umbelliferone-derivatives and no acetylenes. Similar variations were reported in the volatile secondary metabolite composition between the leaves [139] and the whole plant [138] of *A. afra*. The results showed that the oil obtained from the leaves mainly consisted 1,8-cineole (67.4%); while yogomi alcohol (21.6-26.8%) and artemisyl acetate (24.4-32.1%) predominated in the oil extracted from the whole plant.

Activity Reported in the Literature

The scientific research in determining the activity of *A. afra* for its medicinal properties and the publications thereof are given in this section.

Anti-fungal and anti-bacterial

Recent studies have demonstrated that steam distilled *A. afra* oil possess antimicrobial [140] properties. The author report that out of 25 bacterial species and three filamentous fungi used to assess the anti-microbial properties, 15 test bacteria and one fungus showed high degree of inhibition of growth caused by volatile oil. The most susceptible organisms were *Acinetobacter calcoaceticu, Beneckea natrienges, Brevibacterium linens, Brochothrix thermosphacta, Citrobacter freundii, Klebsiella pneumonia* and *Serratia marcescens.*

Trypanocidal and cytotoxic

Nibert and Wink [34] studied *in vitro* effects on antitrypanosomal and cytotoxic activities using *T. b. brucei* and human leukaemia cell, HL-60 against standard drug diminazene aceturate. The IC_{50} (concentration at which 50% of the growth of cells is inhibited) and SI (Selectivity Index, which is the ratio of cytotoxicity of drug against HL-60 to its activity against *T. b. brucei*) for *A. afra* are given in Table 8.

The biological activity was attributed to the major compounds of the extract viz., epolylinalol (29.10%) and dihydrocostunolide (22.14%). However, davonone, bornyl acetate, 4-terpineol and chamazulene were reported to be the major essential oil compounds of the plants [29]. Besides this, the author's do not rule out the possibility of trypanocidal activity due to the presence of other non-volatile compounds. The authors propose that the weak selectivity indices of 4.87 and 1.71 for dichloromethane (DCM) and methanol (MeOH) extracts of the plant against HL-60 warrant its toxicity to human cells.

Anti-diabeteic

The studies reported in literature are either survey or animal studies. Erasto et al. [145] adopting the method of general conversation and questionnaires with the traditional healers and herbalists, obtained

ethno-medical information in the various locations in the Eastern Cape Province in South Africa consisting of many villages classified as rural and poor in the treatment of diabetes. Their studies revealed that 14 species belonging to six families were frequently used. Plants from the family *Asteraceae* were most commonly used in the treatment of diabetes constituting 50% of the plant. Infusion of leaves or roots of *A. afra* was mixed with sugar to mask the bitterness and consumed for a long period on daily basis.

Anti-cancer

The potential of using natural products as anti-cancer agents was recognized by the U.S. National Cancer Institute (NCI) in 1950s and since then been contributing to the discovery of naturally occurring anti-cancer agents [146]. With the discovery of vinca alkaloids, vinblastine and vincristine and isolation of the cytotoxic podophyllottoxins from plant sources in 1950s, more plants were screened for anti-cancer agents. As a result, the US NCI initiated an extensive plant collection program to potentially lead them to the discovery of novel chemotypes showing a range of cytotoxic activities [147] in 1960s. Over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms [148,149]. However, Cragg & Newman [148] report that there is no plant derived clinical anti-cancer agents as yet reached the stage of general use, but a number of agents are in pre-clinical development. A collaborative research programme between US NCI and South Africa Council for Scientific & Industrial Research (CSIR) initially screened 7500 randomly selected plant extracts representing 700 taxa for anti-cancer activity against three human cell lines namely breast MCF7, renal TK10 and melanoma UACC62 and *A. afra* was one of the 32nd plant extracts to have exhibited potent anti-cancer activity [150]. Further, it was screened against 60 human cancer cell lines organized into sub-panels representing leukemia, melanoma and cancer of the lung, colon, kidney, ovary and central nervous system [31]. The anti-cancer activity for plant extract was labeled moderate when the Total Growth Inhibition (TGI – drug concentration that is indicative of the cytostatic effect of the test agent) was observed in the range of 6.25-15 µgm/mL for atleast two cell lines. The DCM–MeOH (1:1 ratio) *A. afra* leaf extract had 26.62 µgm/mL, 15.00 µgm/mL and 9.73 µgm/mL for Renal TK10, Breast MCF7 and Melanoma UACC62 cancer cell lines against standard Etoposide as a positive control (Renal TK10: 27.00 µgm/mL; breast MCF7: >100 µgm/mL and melanoma UACC62: 36.20 µgm/mL). *A. afra* leaf extract was further tested for selective cyto-toxicity over a defined range of concentrations to determine the relative degree of Growth Inhibition (GI_{50}) against each cell lines namely leukemia (L) lines [CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226], non-small cell lung cancer(NSCLC) lines [A549/ATCC, EKVX, HOP-62, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522], colon cancer (CL) lines [COLO205, HCT-116, HCT-15, HT29, KM12, SW-620], central nervous system cancer (CNSC) lines [SF-268, SF-295, SF-539, SNB-19, U251], melanoma (M) lines [LOX IMVI, M14, SK-MEL-2, SK-MEL-28, SKMEL-5, UACC-257, UACC-62], ovarian cancer (OC) lines [IGROV1,OVCAR-3, OVCAR-5, OVCAR-8, SK-OV-3], renal cancer (RC) lines[786-0, A498, ACHN, CAKI-1, SN12C, TK-10, UO-31], prostate cancer (PC) lines [PC-3, DU-145] and breast cancer (BC) lines [MCF7,NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDAN, BT-549]. The log GI_{50} value for *A. afra* extract was 1.02 M and the TGI (µgm/mL) for three most active cell lines were 13.49 (NSCLC NCI-H522); 13.49 (melanoma SK-MEL-5); 14.13 (colon HT29). Fouche et al. [31] research group conclude from their study that the leaf extract of *A. afra* plant to exhibit moderate anti-cancer activity. However, it can provide leads for the development of novel anti-cancer agents.

It is reported that the flavonoids present in the *A. afra* to have chemo-preventive activity against skin cancer (e.g. apigenin); inhibitory effects on chemically induced mammary gland, urinary bladder and colon carcinogenesis in laboratory animals (e.g. hesperetin); and anti-carcinogenic and platelets anti-aggregatory effects (e.g. quercetin) [151,152]. Furthermore, the flavonoid luteolin has been shown to exhibit anti-mutagenic and anti-tumorigenic activities [153].

Cardiovascular

The effect of *A. afra* Jacq. ex. Willd herb on isoproterenol (ISO)-induced myocardial injury in male albino rats of Wister strain was investigated by Sunmonu and Afolayan [35]. Pretreatment with the aqueous leaf extract of the plant at 100 and 200 mg/kg body weight for 30 days prevented the elevation of serum marker enzymes namely lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in myocardial injured rats. ISO-induced animals exhibited decreased levels of glutathione reductase (GR), glutathione peroxides (GPx), superoxide dismutase (SOD) and glutathione (GSH) in the heart, which were restored to near normal levels following treatment with the herb. The extract also attenuated lipid peroxidation (LPO) in the heart and restored the lipid profile to near normalcy, an improved the atherogenic index. The effect was more prominent at 200 mg/kg body weight. Authors suggest that the aqueous extract of *A. afra* exerts cardio protective antihyperlipidemic and antioxidant activities by synthesizing endogenous antioxidants in ISO-induced myocardial injury.

Guantai & Addae-Mensah [154] investigated the cardiovascular effects of a mixture of long chain fatty esters ($C_{44}H_{88}O_2$) and scopoletin isolated from *A. afra* and an aqueous extract of the plant in rabbits. They found that the long chain fatty esters induced hypotensive effects at doses of 0.5, 1.0, 1.5 and 3 mg/kg. The diastolic pressure was affected more than the systolic. Aqueous *A. afra* extract (10-45 mg/kg) had a hypotensive effect *in vivo* and a dose-dependent biphasic effect on the heart *in vitro*. Lower doses induced an initial cardio-stimulation followed by cardio-depression, whereas higher doses were mainly cardio-depressant. Scopoletin, a coumarin derivative, at a dose of 1.0-2.5 mg, induced a dose-dependent decrease in inotropic activity plus an appreciable decrease in chronotropic effects, especially at higher dose levels. These results suggest that *A. afra* and its constituents are potentially useful for the management of hypertensive conditions.

Respiratory infections

The synergistic antimicrobial effects of *A. afra* essential oil when combined with other essential oils obtained from *Agathosma betulina*, *Eucalyptus globulus*, and *Osmitopsis asteriscoides* were investigated by Suliman et al. [58] against *M. catarrhalis* ATCC 23246, *K. pneumoniae* NCTC 9633, *E. faecalis* ATCC 29212, *C. neoformans* ATCC 90112 test organisms by estimating Fractional Inhibitory Concentration using MIC data [155]. The modified version of FIC Index (FIC_{index})

was adopted from Odds [156] that included an additive interpretation [157,158] were determined using following equations:

$$FIC_{index} = FIC_{I} + FIC_{II}$$

where FIC_{I} and FIC_{II} are calculated as follows

$$FIC_{I} = \frac{MIC(A+B)}{MIC(A)} = X$$

$$FIC_{II} = \frac{MIC(A+B)}{MIC(B)} = Y$$

where "A" represented *A. afra* and "B" represented either *A. betulina*, *E. globules* or *O. asteriscoides*.

The data were further evaluated by plotting isobolograms that considered different ratios at which the two plant samples were combined [155] by taking FIC_{I} and FIC_{II} values as X and Y- axes values respectively. The effect of combination of essential oils were considered synergistic (≤0.5), additive (>0.5-1.0), non-interactive (>1.0-≤4.0) or antagonistic (>4.0). The authors report that the individual oils exhibited moderate antimicrobial activity. The MIC values for *A. afra*, *A. betulina*, *E. globulus*, and *O. asteriscoides* ranged (2.6–9.3), (6.0-16.0), (1.3-8.0) and (0.6-8.0) mg/mL respectively against the selective pathogens used in the study. The combination proportions of essential oils between *A. afra* and other oil was varied from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. They also report that their studies did not show any antagonistic interactions but predominantly found additive (>0.5-1.0) activity. However, combination of *A. afra* oil with *O. asteriscoides* in 8:2 ratio yielded synergistic interaction with FIC value of 0.5. The authors suggest that additive/synergistic effect of combination of essential oils needs to be substantiated to establish efficacy through clinical studies.

Viljoen et al. [142] studied the effects of geographical variation on *A. afra* essential oil content (details discussed under the heading "Geographical variation" of this review) and antimicrobial activity. The antimicrobial activity studies by time-kill methodology using the respiratory pathogens *C. neoformans* and *K. pneumoniae* showed prominent antimicrobial effect within 10 min at 0.75% concentration for *K. pneumoniae* and within 60 min at 1% concentration for *C. neoformans*. Investigations of the four major compounds most abundant in the *A. afra* oil (Artemisia ketone, 1,8-cineole, α- and β-thujone) indicated minimal antimicrobial activity when investigated independently and in various combinations against *K. pneumonia*.

To elucidate the rationale behind burning and then inhaling the liberated smoke for its antimicrobial activity, Braithwiate et al. [41] designed an apparatus to simulate the burning process that occurs in a traditional setting and captured the smoke fractions for analysis and bioassay. Parallelly, extracts of MeOH and acetone as well as the essential oil (for the aromatic species) were also prepared and assayed. The anti-microbial studies revealed that the 'smoke-extract' obtained after burning had lower minimum inhibitory concentration (MIC) values than the corresponding solvent extracts and essential oils. The combustion, acetone and MeOH extracts produced different chromatographic profiles, wherein several compounds noted in the smoke fraction were not present in the extracts, suggesting that the combustion process produced an 'extract' with superior antimicrobial activity and provided *in vitro* evidence for inhalation of medicinal smoke as an efficient mode of administration in traditional healing.

Anti-tuberculotic

To verify the traditional phytotherapeutic usefulness of *A. afra* extracts in tuberculosis, Ntutela et al. [159] investigated if *M. aurum* and *M. tuberculosis* replication could be controlled. The authors used aqueous-, MeOH- and DCM extracts of *A. afra* and found that the bacterial replication was inhibited in the *M. aurum* cultures by DCM extract only. Activity of the DCM extract was confirmed in dose-dependent studies against both *M. aurum* and *M. tuberculosis* with an IC_{50} = 270 µgm/ml and IC_{50} = 290 µgm/ml, respectively. Fractionation of the DCM extract and evaluation of its in vitro antimycobacterial activity was found to be mostly associated with isolate fraction C8 that contained several sesquiterpene lactones, the most prominent of which were Artemin and Arsubin. Evaluation of the bactericidal efficacy *in vitro* showed that isolate fraction C8 reduced replication of *M. aurum* and *M. tuberculosis* in a dose-dependent manner with IC_{50} = 1.9 µgm/ml and IC_{50} = 2.0 µgm/ml, respectively, and an MIC = 10 µgm/ml. Further, isolate fraction C8 and the DCM extract were administered to *M. tuberculosis*-infected mice at a tolerated dose of 1000 µgm/kg for up to 26 weeks and mycobacterial burdens compared to untreated-, INH/RIF treated- and aqueous-extract-treated animals to assess its *in vivo* bactericidal activity. Bacterial replication remained unaffected during treatment with either isolate fraction C8 or the DCM extract resulting in pulmonary and splenic bacilli burdens comparable to that of untreated mice. In contrast, INH/RIF (Isonicotinic Hydrazide / Rifampin) treatment cleared *M. tuberculosis* infection after only 8 weeks to undetectable levels. Interestingly, treatment of *M. tuberculosis*-infected mice with aqueous extract of *A. afra* regulated pulmonary inflammation during early infection notwithstanding its inability to inhibit mycobacterial growth. Their study clearly demonstrated that *A. afra* contains *in vitro* anti-mycobacterial activity, modulated pulmonary inflammation in early mycobacterial infection, and that the mouse experimental tuberculosis model could serve as a useful assay for evaluating the utility of phytotherapy. Studies carried out by Mativandelela et al. [32] also supported the traditional use of *A. afra* in TB-related symptoms. The MIC against *M. smegmatis* were in the range of 0.781 to 6.25 mg/mL.

Anti-malarial

A. afra has been used as an infusion to treat malaria in the southern parts of Africa. Clarkson et al. [160] studied 134 species of plants native to South Africa representing 54 families for *in vitro* anti-plasmodial activity against *P. falciparum* strain D10 using the parasite lactate dehydrogenase (pLDH) assay to identify the potential sources of new antimicrobial. Of the species assayed, 49% showed promising anti-plasmodial activity (IC_{50} ≤ 10 µgm/mL) while 17% were found to be highly active (IC_{50} ≤ 5 µgm/mL). The IC_{50} value for *A. afra* leaf extract in (i) DCM, (ii) DCM/MeOH (1:1), (iii) MeOH and (iv) Water were 5, 7.3, 8 and >100 indicating non-polar solvent DCM extract to have highest activity against the *P. falciparum*, substantiating the activity reported earlier by Kraft et al. [161]. Liu et al. [162] investigated the antiplasmodial activity of various extracts of *A. afra* and *A. annua* including an ethnopharmacological prepared sample by using multivariate data analysis. The extracts were tested for activity against *P. falciparum* 3D7 (chloroquine-sensitive strain) with chloroquine, quinine and artemisinin as positive controls. The apolar fractions of both *A. afra* and *A. annua* showed activity against *P. falciparum*

while activity were only found in the tea infusion of *A. annua*. The authors concluded that there aren't any *in vitro* activity in the tea infusion (polar extract) of *A. afra*. Similar conclusions were drawn by Kraft et al. [160] with lipophilic extracts (apolar) of the aerial parts of *A. afra* in the *in vitro* studies. The *A. afra* extract were found to be most active against the chloroquin-sensitive strain PoW and against the chloroquine-resistant clone Dd2 of *P. falciparum* when evaluated with *Cussonia spicata* (Araliaceae), *Vernonia colorata*, *V. natalensis* (Asteraceae), *Parinari curatellifolia* (Chrysobalanaceae), *Clutia hirsuta*, *Flueggea virosa*, (Euphorbiaceae), *Adenia gummifera* (Passifloraceae) and *Hymenodictyon floribundum*, (Rubiaceae). Bioassay-guided fractionation of the extract of *A. afra* yielded seven flavonoids, of which acacetin, genkwanin and 7-methoxyacacetin showed *in vitro* activity; the IC_{50} values ranged from 4.3-12.6 µgm/mL. In addition, several sesquiterpene lactones could be obtained from the most active fractions. Whereas eudesmaafraglaucolide proved to be inactive, the guaianolides 1-desoxy-1alpha-peroxy-rupicolin A-8-O-acetate, 1alpha, 4alpha-dihydroxybishopsolicepolide and rupicolin A-8-O-acetate revealed *in vitro* anti-plasmodial activity.

Anti-spasmolytic

Mulatu and Mekonnen [163] tested the ethanol and aqueous extracts of *A. afra* and leaf of *A. rehan* (from powdered dried leaf and root) on isolated mouse duodenum (MD) and guinea pig ileum (GPI). They tested different concentrations of each extract of the plants ranging from 20-200 µgm/ml in the presence of agonist control, acetylcholine (in MD) and histamine (in GPI) as contraction stimulators. They conclude that *A. afra* leaf ethanol (ALE) and *A. rehan* leaf ethanol (RLE) significantly reduced both spontaneous rhythmic and agonist-induced contractions of MD and GPI. ALE and RLE caused mean contractile response of 44.3 (±0.9% at a dose of 160 µgm/ml) and 35 (± 1.8% at a dose of 120 µgm/ml) respectively in isolated MD and a mean contractile response of 60.9 (±2.7%) and 43.5 (±2.7%) respectively at maximal doses of 200 µgm/ml in isolated GPI; thus justifying the traditional use of these plants in stomach pains and intestinal cramps.

Anti-histaminic and narcotic analgesic

A. afra has been reported to contain anti-histaminic and narcotic analgesic effects [53,124].

Anti-oxidant

The volatile oil from *A. afra* is shown to have exerted considerable anti-oxidative effect [140]. The antioxidant activity of the oil in preventing the discoloration of β-carotene and linoleic acid is given in the Monograph [124]. The free radical 'OH scavenging of the essential oils from *A. afra* was studied by Burits et al. [29] using an assay for non-enzymatic lipid peroxidation in liposome. The IC_{50} value for *A. afra* essential oil were 0.09 µL/mL against pure chamazulene, which were 0.0021 µL/mL, could be ascribed to chamazulene content present in the oil.

In a novel veterinary application, Naidoo et al. [42] investigated the anti-oxidant property of *A. afra* extract in poultry. They attempted to control *Eimera* parasite infections associated with coccidal infection with lipid peroxidation of the intestinal mucosa for economic reasons and to avoid potential dangers of anti-microbials in producing animal protein. *A. afra* extract at 150 mg/kg resulted in feed conversion ratios similar to totrazuril (standard drug) and hence recommended its use as prophylactic and in the management of coccidiosis in poultry industry.

Preservative

Preservation of any product is an important integral part of product development. Generally, a combination of preservatives is used for wide spectrum antimicrobial activity. Use of natural plant products is generally considered to be safe in comparison to synthetic preservatives. The preservative use of aromatic essential oils in part or full in cosmetic preparations not only prevents the product from microbial spoilage but also enhances dermato-cosmetic properties [164]. Muyima et al. [165] evaluated the preservative capabilities of the essential oils obtained from *A. afra* and others viz., *P. incana*, *L. officinalis* and *R. officinalis* in aqueous cream by Challenge Test against seven micro-organisms namely *E. coli* ATCC 35218; *S. aureus* ATCC 2592; *P. aeruginosa* ATCC 27853; *C. albicans* ATCC 10231; *A. niger* ATCC 16404; and two environmental isolates identified as *P. aeruginosa* and *R. pickettii* by Challenge Test. The concentration of essential oils in aqueous cream was 0.5, 1.0 and 1.5% v/w of individual oil as sole preservative and the control cream contained commercial preservative. Their studies show that the antimicrobial property of essential oils in the test creams in all the three concentrations were better than the control cream except *P. incana* which were almost similar to that of control cream. The Challenge Test in almost all test creams showed $\log_{10}$ reductions within 24 hrs, two to three $\log_{10}$ reductions in 2 days and four $\log_{10}$ within 2-7 days, suggesting their use as natural cosmetic preservatives.

Ashebir and Ashenafi [166] assessed the *in vitro* antibacterial activity of *A. afra* leaves traditionally used in the food borne diseases. The growth or inhibition of micro-organisms like *B. cereus*, *S. aureus*, *S. boydii*, *S. flexineri*, *S. typhimurium* and *E. coli* were determined in culture media using 5% weight by volume crude extract of *A. afra* leaves in distilled water. Their results showed that *B. cereus* and *S. aureus* had markedly lower final counts in the media containing crude preparation when compared to Control (without the crude extract). Retarding effect were noted on *S. Flexineri* and *S. Boydii* in the initial stages. The counts of *S. typhimurium* were as low as one log unit against the Control until eight hours while it had no effect on *E. coli*. Hence, the authors suggested of taking extract at four hours intervals to enhance the anti-microbial effect.

Insecticide

The volatile oil obtained from the ground parts of the crop showed antimicrobial activity against various bacteria and fungi of public health or agricultural significance [124].

A. afra is also known to have good insecticidal properties and can be used as a companion plant to reduce pest pressure on crops. It is planted as a border plant surrounding other medicinal or vegetable plants. It is used in formulations for animal shampoos and insect repellents [124].

Analysis and Quality Control

Avula et al. [44] developed a simple and specific High Performance Liquid Chromatography (HPLC) technique to determine flavonoids viz., (i) apigenin, (ii) chrysoeriol, (iii) tamarixetin, (iv) acacetin and (v) and genkwanin (Figure 8) in the aerial parts of the 11 samples of *Artemisia afra* Jacq. ex Willd plant obtained from widely separated populations in the provinces of Kwa-Zulu Natal and the Western Cape in South Africa. They also validated the technique for accuracy and precision before undertaking quantitative analysis. The limits of detection (LOD) by HPLC-MS were found to be 7.5, 7.5, 10, 2.0, and

2.0 ng/mL; and by HPLC-UV were 500, 500, 500, 300, and 300 ng/mL for apigenin, chrysoeriol, tamarixetin, acacetin and genkwanin, respectively. The limits of quantification (LOQ) by HPLC-MS were found to be 25, 25, 25, 10 and 10 ng/mL; and by HPLC-UV were 1000, 1000, 1000, 500 and 500 ng/mL for apigenin, chrysoeriol, tamarixetin, acacetin and genkwanin, respectively. They reported HPLC-MS method 50-150 times more sensitive compared to HPLC-UV method. The flavonoid contents estimated by HPLC-UV and HPLC-MS are presented in Figure 9 from the calculated average values (11 samples of the plant) along with the standard deviations thereof for each component expressed in percentage. It was seen that irrespective of the method (which differs in sensitivity by many hundred folds), the average values and the standard deviation values were almost same and the compound Tamarixetin was absent in *A. afra* when determined by both methods. The same trend were seen when individual HPLC-UV analytical values were compared with HPLC-MS in each plant sample (Figure 9). Also, that the standard deviation values quoted by the authors for each triplicate samples analyzed for all the 11 plant samples by either method were shown to be higher than the average values, the reasons of which are not being given or well understood.

Pharmaceutical Dosage Form

Research achievements has not yet reached to an extent that a successful dosage form of *A. afra* is produced at industrial scale and made available for mass consumptions. The plant is still being screened for its various potential pharmacological properties and some positive results have been achieved. Besides, the available literature does not report separation and isolation of various chemical constituents & screened individually for the activity. However, there could be every possibility to hope that some parallel ongoing synthetic work is in full progress that would be made public with filing of the patent for product and process.

Some work has been done at the University of the Western Cape, South Africa in an attempt to develop a pharmaceutically acceptable dosage form. Komperlla [167] attempted to formulate and evaluate tablets from *A. afra* plant. From the studies, the author concluded that the dried aqueous extract of *A. afra* leaves were problematically very hygroscopic. However, tablets of suitable pharmaceutical quality could be manufactured from the dried extract of *A. afra* leaves under controlled humidity conditions. Dube [39] aimed at preparing a standard tea bag dosage form from standardized *A. afra* leaves and from the freeze-dried aqueous extract to minimize dose variation and evaluated the dosage form criteria. He concluded from his studies that the (i) tea bag were a suitable dosage form for *A. afra* standardized dried leaves but not the freeze-dried aqueous extract powder due to stability problem and (ii) tea-bag preparations did not have similar infusion profiles to that of loose leaves, but could be still used if adjustment in the dose preparation and administration methods are made.

Toxicity

The herb, *A. afra* is not patented for its being used traditionally for number of ailments. This could be one of the reasons why this herb could not attract so much of attention from the industry and validated scientifically through clinical trials [168,169].

Safety of *A. afra* has been a controversial issue due to its high thujone content. In 1970s, WHO declared the plant being unsafe for consumption but however its use in folklore medication, is gaining significance in use of modern diseases. Oyedeji et al. [43] studied the α-thujone content isolated from essential oil obtained by hydrodistillation of fresh and dried twigs of *A. afra* plants obtained from different locations in the Eastern Cape, Free State and KwaZulu-Natal. Analysis of the oil by GC and GCMS revealed compositional variations in the levels of α- and β-thujone, 1,8-cineole and camphor in the extracts. They also found that the concentration of α-thujone increased significantly in the dry leaves when compared with the fresh leaves. Based on these results, they suggested the use of fresh leaves for infusion.

Mukinda and Syce [170] investigated the safety of *A. afra* aqueous extract (mimicking the traditional decoction dosage form) by determining its pharmaco-toxicological effects after acute and chronic administration in mice and rats, respectively. In mice, single intraperitoneal injections of the extract (1.5-5.5 gm/kg) induced a regular dose-dependent increase in the death rate and incidence of general behaviour adverse effects, while with single oral doses (2-24 gm/kg) increase in the incidence of general behavioral adverse effects and mortality rate were dose-independent. The LD_{50} after acute intraperitoneal and oral doses were 2.45 and 8.96 gm/kg, respectively. Rats given oral doses of the extract (0.1 or 1 gm/kg/day) survived the 3 months of dosing (i.e. LD_{50} much higher than 1mgm/kg), experienced no significant changes in general behaviour and haematological and biochemical parameters, except for transient decrease in AST activity. No significant changes were observed in organ weights, and histopathological results showed normal profile suggesting no morphological alterations. They concluded that the *A. afra* extract is non-toxic when given acutely and has low chronic toxicity potential; in high doses it may have a hepatoprotective effect.

The Directorate Agricultural Information Services [133] instructs the users that *A. afra* should not be taken longer than a period of 7 to 10 days as it can cause headaches and shaking, owing to high content of thujone. Also, this oil must not be used internally, and should be used with caution during pregnancy and in epilepsy.

Clinical Studies

An effort was made by van Wyk [171] team of researchers from the University of Western Cape in South Africa in 2003-04. A protocol entitled "A Pilot study on Mild to Moderate Asthmatic subjects to test the bronchodilatory effect of the herbal plant *Artemisia afra* was prepared to undertake clinical study to evaluate the efficacy and safety of *A. afra*" herbal preparation for asthma. The protocol was designed after incorporating various aspects given in the Guidelines of different Countries including WHO by thoroughly going through the Guidelines available on the web between Feb. to Aug. 2003. They submitted the Protocol to the Medical Control Council and Ethics Committees which was rejected on account of lack of safety data, toxicological studies and pharmacokinetics of the drug. The researchers however feel that permission to undertake clinical study should be considered on the basis of historical use of herb to circumvent the issue of lack of safety data.

In this context, it is pertinent to mention the recent efforts made at global level in the direction of conduct of Clinical Trials so as to prevent the patients from self-medication with unregulated products raising number of safety concerns that exists for lack of specific Guidelines to conduct Clinical Trials in herbal/traditional medicines. As per the Innovations Report [172], world's first clinical study on

African traditional medicine will be undertaken by The International Center for Indigenous Phytotherapy Studies (TICIPS) in collaboration with the University of Missouri-Columbia and the University of the Western Cape, South Africa. The center will be funded by a $4.4 million, 4-year grant from the National Center for Complementary and Alternative Medicines (NCCAM), a division of the National Institutes of Health. On the 7th International Clinical Trials Day 2011, a Multi-disciplinary University Traditional Health Initiative (MUTHI) which is new international consortium with the aim of increasing the capacity of African clinical and public health researchers to conduct trials of traditional medicines was launched by Prof. Quinton Johnson, Director of the South African Herbal Science and Medicine Institute at the University of the Western Cape outlining its plans to facilitate the assessment of the medicinal properties of plants [173]. With an intension of "one-world medicine" for the sake of all patients in industrialized and developing countries, Efferth [174] discussed strategies for (i) preservation of traditional knowledge on natural medicines, (ii) sustainability of medicinal herbs and natural products, and (iii) standardization and quality control.

Conclusion

From the available literature, it can be stated that *A. afra* is a potential herb showing activity for many ailments. The capabilities Mother Nature has imbibed in this plant has been only attempted and explored in past few years, needs to be accelerated in all the areas so that successful products are available for mass consumption to alleviate diseases afflicting mankind. This review is a humble effort to compile the existing literature in one paper, covering maximum aspects of *A. afra* with a hope to benefit the researchers.

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