World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:11, No:6, 2017

Evaluation of South African Plants with Acaricide Activity against Ticks

G. Fouché, J. N. Eloff, K. Wellington

***Abstract*—**Acaricides are commonly used to control ticks but are toxic, harmful to the environment and too expensive to resource-limited farmers. Traditionally, many communities in South Africa rely on a wide range of indigenous practices to keep their livestock healthy. One of these health care practices includes the use of medicinal plants and this offers an alternative to conventional medicine. An investigation was conducted at the CSIR in South Africa, and selected indigenous plants used in communities were scientifically evaluated for the management of ticks in animals. 17 plants were selected from 239 plants used traditionally in South Africa. Two different organic extracts were prepared from the 17 samples, resulting in 34 plant samples. These were tested for efficacy against two tick species, namely *Rhipicephalus microplus* and *Rhipicephalus turanicus.* The plant extracts were also screened against Vero cells and most were found to have low cytotoxicity. This study has shown that there is potential for the development of botanicals as natural acaricides against ticks that are non-toxic and environmentally benign.

Keywords*—Rhipicephalus microplus*, *Rhipicephalus turanicus*, ticks, plant extracts, South Africa.

I. Introduction

At present, livestock productivity in Africa is struggling to keep pace with the growing demands of an ever increasing human population. Africa accounts for a share of 22% of the global market [1]. The overall share of the livestock industry in Africa's GDP is 5% for South Africa and between 25% and 30% for the underdeveloped countries in the region, such as Kenya; Uganda; and other south, north, and east African countries. Nearly 70% of the population in these countries own livestock, including pastoralists living in arid and semi-arid zones. Of these, nearly 200 million people rely on livestock for income. Thus, livestock plays a pivotal role in providing nutrition for a majority of the population in Africa [1].

Parasites and animal diseases are major threats to the African livestock industry. The major parasites that infect animals include ectoparasites (ticks, mites, lice, and fleas) and endoparasites (tapeworms, protozoa, trematodes or flukes, and nematodes). Owing to the high cost of parasiticides, many farmers in Africa use natural plant-based medicines to protect and treat their livestock population against diseases [1]. Moreover, the use of plant-based medicines continues to grow as there is no resistance reported with the use of these medicines in veterinary practice.

Tick-borne diseases are regarded as one of the major impediments to efficient livestock production across Africa. Based on prevalence and economic impact, some of the major tick-borne diseases in the Southern African Development Community (SADC) region are bovine anaplasmosis, bovine babesiosis, Heartwater, African swine fever, bluetongue, and East Coast Fever. Ixodid ticks such as *Amblyomma hebraeum*, *Rhipicephalus decoloratus, Rhipicephalus turanicus* and *Rhipicephalus microplus* in particular are prevalent in SADC [2].

The African tick, *Rhipicephalus* is a tick species found in South Africa and the most vital constrains to cattle have been ticks and tick-borne diseases [3], [22]. The African blue tick, *Rhipicephalus decoloratus*, transmits African redwater through a protozoal parasite, and tick-borne gall sickness through a bacterial parasite. Blue ticks prefer cattle but will feed on horses, donkeys, sheep and goats and is a one-host parasite [1]. The appearance of the two ticks, *R. turanicus* and *R. sanguineus* is very similar and it is difficult to distinguish between them. This is probably mainly due to the widespread distributions of both of these ticks. *R. turanicus* is found in the Gauteng and KwaZulu-Natal Provinces of South Africa [1].

Amitraz, ivermectins, and pyrethroids are the most commonly used acaricides for the prevention of tickborne diseases in the global livestock industry. However, over the years, ticks have become increasingly resistant to these acaricides. Many researchers state that the severity of tick resistance has reached a level where resistance must be expected in ticks within five to ten years of introduction of any new type of acaricide [1].

For many decades, medicinal plants have been utilized as the principal source of prevention and control of livestock diseases. Rural poultry farmers largely focus on ethnoveterinary medicine (EVM) due to the lack of veterinarians in rural areas. High cost of allopathic medicine is also an important factor for farmers so they rely on EVM. Plant-based parasiticides are the safest options for industry players to ensure 100% organic nature of their product output.

There are more than 68,000 plant species in Africa, of which nearly 35,000 are known to have some medicinal properties. Medicinal plants and the drugs derived from them form a crucial part of the healthcare remedies for rural people in Africa. There are more than 3,000 medicinal plant species in Southern Africa itself, of which nearly 300 species have a common use in the traditional healthcare system in Africa [1]. The aim of this project was to investigate and produce new,

G. Fouche is with the Biosciences Unit, Council for Scientific and Industrial Research (CSIR), Pretoria 001 RSA (e-mail: gfouche@csir.co.za).
J. N. Eloff is with Department of Paraclinical Sciences ,University of Pretoria, Onderstepoort, 0110 RSA (e-mail: kobus.eloff@up.ac.za).
K. Wellington is with the Biosciences Unit, CSIR, Pretoria 0001 RSA, (e-mail: kwellington@csir.co.za).

natural and safe ingredients from indigenous South African plants for the effective management of ticks in animals.

II. Materials and Methods

A. Plant Material Collection

17 South African plants species (*Aloe rupestris, Antizoma angustifolia, Calpurnia aurea, Senna italica* subsp *arachoides* (Burch.) Lock, *Cissus quadrangularis, Clematis brachiata, Clematis villosa, Cleome gynandra, Ficus sycomorus, Gnidia deserticola, Hypoxis rigidula, Maerua angolensis, Monsonia angustifolia, Pelargonium luridium, Schkuhria pinnata, Sclerocarya birrea* and *Tabernaemontana elegans*) were collected and voucher specimens were submitted to the South African Biodiversity Institute in Pretoria, South Africa.

B. Production of Plant Extracts

Collected plant material was dried in an oven at 30-60 ºC and hammer milled to obtain fine ground plant material. Two different organic solvents were used to prepare extracts; ethanol and acetone, from each of the 17 plant species. The solvent extracts were prepared by pouring 200 ml of solvent onto 20 g ground plant material which was then stirred for 1 h. The residue was re-extracted with the same amount of solvent for 1 h and for the third time, the same volume of solvent was used but the mixture was stirred overnight. The extracts were combined and the ethanol and acetone evaporated using a rotary evaporator.

C. Adapted Shaw Larval Immersion Test (SLIT)

Fully engorged females of *R. microplus* (Acari: Ixodidae) were obtained from Clinvet International, Bloemfontein, South Africa. The strain used is susceptible to an organophosphate acaricide, chlorfenvinphos, at a field concentration of 300 ppm. The ticks were placed into 5 conical flasks containing 20 females each. The flasks were incubated at 26 ± 2 °C at a relative humidity of greater than 70% for oviposition and hatching. Testing was performed between 17 and 25 days post hatching.

The test, originally described by Shaw was used and modified to increase the period of larval incubation after treatment to 72 h before the test was read [4]. A 1% w/v extract dilution was prepared and 0.1 g of extract was weighed and diluted in 10 ml of distilled H_2O containing 0.02% Triton X-100 and 1% acetone (diluent). After dilution, the solution was vortexed for 1–10 min depending on solubility and then put in an ultrasonic water bath for 10 min to dissolve the extract in the diluent. Some extracts did not dissolve completely and was used as a suspension.

Approximately, 200 larvae between 16 and 25 days post hatching were placed between two round Whatman no 1 filter papers (diameter 120 mm) to form a larvae sandwich. It was then placed in a pie plate (diameter 140 mm) and ten ml of a 1% solution from the plant extract was poured over the tick sandwich to expose larvae to the solution. Each run also included a positive control (300 ppm – Field concentration of Chlorfenvinphos – Supadip 30% w/v) and a negative control (diluent). After 30 min, excess solution was drained from the filter paper sandwich and it was transferred to a clean filter paper (Whatman no 1, diameter 250 mm). 100 larvae were brushed from each half of the sandwich onto a clean filter paper envelope. It was crimped closed and placed in an incubator at a temperature of 26 ± 2vºC and RH ≥ 70%. After 72 h the number of live versus dead larvae was counted and efficacy of extract to kill the larvae was determined against a negative control sample. Incubation for each plant extract was done in duplicate. The corrected mortality (CM) as well as the mean for each plant extract.

CM was calculated by making use of Abbott's formula i.e.:

$$CM\% = [\%i - \%c/100 - \%c] \times 100$$

where % i = % mortality in test extract; %c = % mortality in negative solvent control (diluent); CM% = corrected mortality

D. Adult Immersion Test (AIT)

Fully engorged females of *R. turanicus* were obtained from Clinvet International, Bloemfontein, South Africa. The contact bioassay described by [16] was employed. 1 µl of a 20% concentration (100 mg/500 µl of acetone) of the extract of each plant species was placed on the dorsum of each of ten ticks (*R. turanicus*) for 1 minute before storing them in a vial covered with a perforated stopper. The same was done for the negative control (distilled water only) and positive control (Cypermethrin 0.55% m/v). Each treatment was replicated three times. The vials were examined hourly for 6 hours after treatment and the findings were recorded. Mortality rate was recorded after 24 hours. Ticks were considered alive if they exhibited normal behaviour (when breathed upon or physically stimulated with a tweezer). Any tick that did not respond to human breath (CO_2) after 30 seconds was considered dead, those showing some difficulty in movement or maintaining a normal posture were termed weak or very weak if there was no leg coordination or ability to right themselves.

The tick mortality rate was calculated according to Chungsamarnyart [17] as follows:

$$\text{Corrected mortality, CM (\%)} = T/C \times 100$$

where T is the number of ticks alive in the treatment group; C is the number of ticks alive in the control group.

E. Determination of the Cytotoxicity of the Plant Extracts

The plant extracts were screened against Vero African Green monkey kidney cells using the tetrazolium-based (MTT) colorimetric assay to determine the cell viability [5]. Each of the plant extracts were redissolved in their extracting solvents (acetone or ethanol) and tested at a concentration range of 1–0.001 mg/ml. Doxorubicin was used as a control. The test was repeated in quadruplicate and the assays were repeated three times. A plant extract having a LC_{50} value less than 20 µg/ml is regarded as toxic.

TABLE I
EVALUATION OF ACARICIDAL AND CYTOTOXICITY ACTIVITY OF PLANT EXTRACTS AGAINST TWO TICK SPECIES

Family	Plant Name	Traditional Use	Reference	Plant Part	Solvent	CM (%) AIT, *R. turanicus*	CM (%) LIT, *R. microplus*	Cytotoxicity (IC_{50} µg/ml)
Asphodelaceae	*Aloe rupestris*	Expels pin worms	[6]	Roots	Acetone	ND	ND	ND
					Ethanol	10	ND	ND
			[6]	Leaves	Acetone	ND	2.4	63.46 ± 11.00
					Ethanol	0	46.1	101.99 ± 3.86
Menispermaceae	*Antizoma angustifolia*	External parasites. Use for ticks on cattle	Verbal communication, [9]	Roots	Acetone	**73**	26.6	43.59 ± 6.28
					Ethanol	**60**	**67.3**	24.30 ± 0.22
Fabaceae	*Calpurnia aurea*	Used for lice and to relieve itches. Destroy maggots in sores on cattle. Treat abscesses.	[7]	Stems	Acetone	**100**	ND	166.63 ± 7.97
					Ethanol	ND	ND	ND
				Leaves and flowers	Acetone	**100**	xxx	166.63 ± 7.97
					Ethanol	ND	**100.0**	504.32 ± 3.90
Leguminosae	*Senna italica* subsp *arachoides*	Anthelmintic. Used to treat influenza, purgatives, stomach complaints and diarrhea, indigestion, liver and gall bladder complaints, gastrointestinal disorders, dysmenorrhoea, uterine pain.	[8]	Roots, leaves and fruit	Acetone	**80**	xxx	46.31 ± 2.89
					Ethanol	ND	**96.7**	550.67 ± 12.49
Vitaceae	*Cissus quadrangularis*	Used for burns, wounds, febrile pain, malaria, induce milk flow in cattle, gastro-intestinal complaints	[6], [7]	Stems	Acetone	ND	**100.0**	41.44 ± 2.96
					Ethanol	ND	**98.9**	74.33 ± 3.68
Ranunculaceae	*Clematis brachiata*	Intestinal worms. Used as enemas for abdominal disorders, rashes, vermifuge and for bots on horses.	[6]	Whole plant	Acetone	**50**	**57.9**	117.00 ± 4.08
					Ethanol	ND	0.0	485.28 ± 21.74
Ranunculaceae	*Clematis villosa*	Roundworms	Verbal communication	Roots	Acetone	ND	ND	45.05 ± 0.93
					Ethanol	ND	**100.0**	76.72 ± 3.04
Capparidaceae	*Cleome gynandra*	Treatment of headaches and stomach aches, epileptic fits and earache. It repels effectively all stages of the livestock ticks *R. appendiculatus* and *A. variegatum*	[10], [11]	Leaves	Acetone	**70**	32.8	553.61 ± 18.83
					Ethanol	**80**	**80.5**	39.51 ± 0.36
Moraceae	*Ficus sycomorus*	Ectoparasites, mental illness, wound dressing and diarrhoea	[12]	Stems	Acetone	**90**	ND	48.74 ± 1.32
					Ethanol	ND	ND	122.45 ± 18.45
				Bark and stems	Acetone	0	22.5	172.94 ± 8.91
					Ethanol	ND	11.6	458.36 ± 7.87
Thymelaeaceae	*Gnidia deserticola*	Malaria and parasites	Verbal communication	Whole plant	Acetone	**70**	**75.6**	89.61 ± 4.76
					Ethanol	**70**	19.5	93.53 ± 6.44
Hypoxidaceae	*Hypoxis rigidula*	Ticks and fleas. Antibacterial activity	[13]	Bulbs	Acetone	ND	**59.9**	64.04 ± 2.53
					Ethanol	ND	25.2	343.43 ± 14.89
Capparaceae	*Maerua angolensis*	Nematodes and parasites, vapour or steam is inhaled to treat children with convulsions	[8], verbal communication	Leaves	Acetone	**100**	**54.3**	73.76 ± 0.27
					Ethanol	30	**100.0**	126.83 ± 5.85
				Stems	Acetone	**50**	ND	73.76 ± 0.27
					Ethanol	ND	ND	ND
Geraniaceae	*Monsonia angustifolia*	Malaria, helminthiasis, anthrax (cattle), diarrhoea (calves and lambs).	[14]	Whole plant	Acetone	ND	33.4	120.37 ± 4.06
					Ethanol	**80**	**52.9**	34.67 ± 0.86
Geraniaceae	*Pelargonium luridium*	Anthelmintic, used to treat diarrhoea, backache and abdominal pain in infants, and dysentery, sick calves, colic, nausea, vomiting and fever.	[6], [8]	Whole plant	Acetone	0	35.0	30.58 ± 3.40
					Ethanol	17	**59.6**	32.86 ± 1.06
Asteraceae	*Schkuhria pinnata*	Malaria, used for eye infections, pneumonia, diarrhoea, heartwater, abortifacient and as a contraceptive.	[7], [15]	Whole plant	Acetone	**90**	4.6	39.93 ± 1.80
					Ethanol	**70**	24.4	89.14 ± 4.14
Anacardiaceae	*Sclerocarya birrea*	Used for malaria, diarrhoea, dysentery, proctitis, prophylactic, fever, stomach ailments, headaches, ulcers, toothache, backache, infertility, constipation, to strengthen the heart, destruction of ticks, menorrhagia, schistosomiasis, sore eyes, heart pain, snake bite and other venoms.	[6], [8]	Bark and root	Acetone	ND	30.0	418.27 ± 7.89
					Ethanol	ND	20.1	486.71 ± 3.11
				Fruit	Acetone	**63**	ND	ND
					Ethanol	30	ND	ND
Apocynaceae	*Tabernaemontana elegans*	Parasites	Verbal communication	Leaves	Acetone	ND	22.3	32.35 ± 0.88
					Ethanol	27	0.8	40.04 ± 4.78

In Table I, LIT is Larval immersion test. AIT is Adult immersion test. LPT is Larval packet test. ND is Not Determined. Xxx means Larvae too mixed up with extract debris to be evaluated. Doxorubicin: 2.97 ± 0.016µg/ml Negative control: diluent (used to prepare extractions). Positive control: 300 ppm (Field concentration of Chlorfenvinphos) Supadip 30% m/v, CM 99.0%. CM is corrected mortality.

F. Statistical Analysis

Determination of the cytotoxicity of the plant extracts was done in triplicate. The results were expressed as mean ± standard deviation (SD). P values of <0.05 were regarded as significant.

III. Results

A. Determination of the Acaricidal Activity

For efficacy testing the larvae of *R. microplus* were exposed to a 1% solution (0.1 g per 10 ml) of each of the plant extracts. The results are shown in Table I. 14 of 34 plant extracts produced mortality greater than 50% with most of it ethanol extracts (10). Ethanol extracts of *C. aurea* (leaves, flowers), *C. quadrangularis* (stems), *C. villosa* (roots) and *M. angolensis* (leaves) had the best activities (CM = 100%). Among the acetone extracts, *C. quadrangularis* (stems, CM = 100%) had the best activity followed by *G. deserticola* (whole plant, CM = 75.6%). For efficacy testing the adults of *R. turanicus* were exposed to 1 µl of a 20% concentration (100 mg/500 µl of acetone) of each of the plant extracts. The results are shown in Table I. 17 of 34 plant extracts produced mortality greater than 50% with most of it acetone extracts (12). Acetone extracts of *C. aurea* (stems, leaves and flowers) and *M. angolensis* (leaves) had the best activities (CM = 100%). Among the ethanol extracts, *C. gynandra* (leaves) and *M. angustifolia* (whole plant) had the best activities (CM = 80%).

B. Determination of the Cytoxicity of the Plant Extracts

The results are shown in Table I. From these results, it is evident that none of these plant extracts could be classified as toxic since their LC_{50} values were greater than 20 µg/ml. For the acetone extracts, *C. gynandra* was the least toxic (LC_{50}= 553.61 µg/ml) followed by *S. birrea* (LC_{50}= 418.27 µg/ml). Amongst the ethanol extracts *Senna italic* subsp *arachoides* was the least toxic (LC_{50}= 550.67 µg/ml) followed by *C. aurea* (LC_{50}= 504.32 µg/ml).

IV. Discussion

Four ethanol extracts namely that of *C. aurea* (leaves, flowers), *C. quadrangularis* (stems), *C. villosa* (roots) and *M. angolensis* (leaves) and two acetone extracts namely of *C. aurea* (stems, leaves and flowers) and *M. angolensis* (leaves) showed excellent activities (CM = 100%). Different activities were observed for the larvae and adult stages of the two tick species. Although differences were observed, two plant species, of *C. aurea* and *M. angolensis* consistently showed potent aricicidal activity against both tick species.

C. aurea is a multi-stemmed, evergreen plant with light open crown belonging to the pea and bean family of trees (Fabaceae). This genus is well spread in most places including forests, hills, bushvelds and other places. In southern Africa, it is well distributed in the Eastern Cape throughout to KwaZulu Natal, Mpumalanga, Swaziland, Gauteng, Limpopo [7]. In South Africa, *C. aurea* is used traditionally to control lice, to treat itches and allergic rashes and destroy maggots in sores on cattle [7]. There is potential for *C. aurea* to be used for tick control since this plant species is found throughout Africa and also has the ability to resist drought and overgrazing. Phenolic compounds were identified as the main chemical constituents of *C. aurea* [18] that are thought to be responsible for the attraction of over twelve species of ticks.

M. angolensis is indigenous to the tropical east of South Africa and Swaziland [8] and belongs to the family Capparaceae. The leaves are used in traditional medicine to treat infections and parasitic diseases as well as diseases of the digestive tract (verbal communication). In South Africa the Venda people use the leaves and bark which are heated over a fire, without water, and the resultant vapour or steam is inhaled to treat children with convulsions [8].

C. quadrangularis is a perennial succulent vine indigenous to Asia and Africa belonging to the Vitaceae family. The green stems are up to 3 cm in diameter and applied to heal sores and wounds in livestock [19]. There are short-lived broad and fleshy lobed green leaves towards the ends of some stems. The plant is drought tolerant but not very frost resistant. It can be found in dry woodland areas in Mpumalanga and Limpopo provinces, but widespread inland in Southern Africa. Several pharmacological activities have been reported for this plant such as bone fracture healing, gastroprotective, antiulcer, antioxidant, analgesic, antiosteoporotic, antihemorrhoidal, anti-inflammatory, anabolic and androgenic, parasympathomimetic, antibacterial, antifungal and anthelmintic activity [6], [7].

G. deserticola belongs to the family Thymelaeaceae and is well distributed in Africa, Arabia, India and Sri Lanka with more than half of the species of this genus indigenous to South Africa [8]. Traditionally, African species of *Gnidia* are used to treat ailments in humans including asthma, backaches, malaria, nightmares, burns, snake bites, headaches, influenza and fevers, constipation, ulcers and a whole range of other conditions [8]. Although *Gnidia* species are used to treat wide range of conditions in humans, severe irritant effects and death among humans and animals have been reported due to the presence of poisonous coumarins and diterpene esters [20]. Chemical investigation showed the *Gnidia* species to contain various groups such as cumarins, flavonoids, lignans, sesquiterpenes, diterpenes, sterols and lipids among other constituents [20].

M. angustifolia is an herb and belongs to the Geraniaceae family. It grows annually and is found in open grasslands across South Africa and tropical Africa. The plant usually grows up to 0.5 m in height and the seeds are usually dispersed by rain in rainy seasons and wind in windy seasons. It is well distributed across the southern Africa region including South Africa, Lesotho, Swaziland, Namibia and

Mozambique [14]. Other traditional uses include the use as a blood cleanser and for the treatment of heartburn, anthrax and diarrhoea [8]. Unfortunately, not much phytochemical investigations have been done on this species or the genus as a whole. However, extracts and compounds isolated from this species have been reported to treat dementia and erectile dysfunction [21].

When comparing the activity of the acetone and ethanol plant extracts against *R. microplus* to that against *R. turanicus*, it is evident that ethanol extracts exhibited a higher potency compared to acetone extracts. Thus, it appears that *R. microplus* is more susceptible to the effects of the active principles in the ethanol extracts.

V. Conclusion

Acetone extracts prepared from the stems, leaves and flowers of *Calpurnia urea* as well as the acetone and ethanol extracts from the leaves of *Maerua angolensis* exhibited excellent acaricidal activity against the tick species, *Rhipicephalus microplus* and *Rhipicephalus turanicus*. These plant extracts showed low toxicity against Vero cells. Further studies, especially *in vivo* evaluation needs to be conducted as well as the isolation and identification of the compounds responsible for the acaricidal activity in these extracts. This will be beneficial for the discovery and development of novel natural acaricides. These plant species could be good alternatives in an integrated control effort against these ticks to overcome problems associated with the use of chemical acaricides.

Acknowledgment

The authors thank the Technology Innovation Agency (TIA) of South Africa for financial support.

References

[1] Markets and Markets, Market study conducted for the CSIR titled "Animal parasiticides market", 2014, pp. 1–51.

[2] A. Bram, Tick-borne livestock diseases and their vectors: The global problem. In: ticks and tick-borne diseases. FAO Animal Production and Health. Food and Agriculture Organization, Rome, 1983, pp. 7–11.

[3] A. Estrada-Pe˜na, A. Bouattour, J. L. Camicas, A. Guglielmone, I. Horak, F. Jongejan, A. Latif, R. Pegram, A. R. Walker, 2006. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Exp. Appl. Acarol*. 2006, 38, 219–235.

[4] R. D. Shaw, M. Cook, R. E. Carson, Developments in the resistance status of the southern cattle tick to organophosphorus and carbamate insecticides. *J. Econ. Entomol*. 1968, 61, 1590–1594.

[5] L. J. McGaw, V. Steenkamp, J. N. Eloff, Evaluation of *Athrixia* bush tea forcytotoxity, antioxidant activity, caffeine content and presence of pyrrolizidinealkaloids. *J. Ethnopharmacol*. 2007, 110, 16–22.

[6] A. Hutching, A. H. Scott, G. Lewis, A. Cunningham, *Zulu Medicinal Plants: An Inventory*. University of Natal Press, Scottsville, 1996.

[7] L. J. McGraw, J. N. Eloff, Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *J. Ethnopharmacol.* 2008, 119, 559-574.

[8] B. Van Wyk, B. van Oudtshoorn, N. Gericke, Medicinal Plants of South Africa (2nd Ed). Briza Publications, Pretoria. 2009.

[9] H. De Wet, F. R. van Heerden, B van Wyk, Alkaloids of *Antizoma angustifolia, Biochem. Systematics and Ecol.* 2004, 32, 1145–1152.

[10] R.T. Narendhirakannan, S. Subramanian, M. Kandaswamy, Anti-inflammatory and lysosomal stability actions of *Cleome gynandra* L. studied in adjuvant induced arthritic rats, *Food and Chemical Toxicology*, 2007, 45, 1001–1012

[11] S. Nyalala, Brian Grout African spider flower (*Cleome gynandra L./Gynandropsis gynandra* (L.) Briq.) as a red spider mite (*Tetranychus urticae* Koch) repellent in cut-flower rose (*Rosa hybrida* L.) cultivation, *Scientia Horticulturae*, 2007, 114, 194–198.

[12] U.K. Sandabe, P.A. Onyeyili, G.A. Chibuzo, Phytochemical screening and effect of aqueous extract of *Ficus sycomorus* L. (Moraceae) stembark on muscular activity in laboratory animals, *J. Ethnopharmacol.*, 2006, 103, 481–483.

[13] D. Van der Merwe, G. E. Swan, C. J. Botha, 2001. Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the North West Province of South Africa. *J South African Veterinary Association,* 2001, 72, 189–196.

[14] E. Van Koenen, Medicinal, Poisonous and Edible Plants in Namibia. Klaus Hess Publishers, Windhoek. W.-K. Chen, *Linear Networks and Systems.* 2001.

[15] V. Steenkamp, Traditional herbal remedies used by South African women for gynaecological complaints. *J. Ethnopharmacol*, 2003, 86: 97-108.

[16] A. Zorloni, Evaluation of plants used for the control of animal ectoparasitoses in Southern Ethiopia (Oromiya and Somali Regions). Magister Scientiae Thesis, University of Pretoria.2008.

[17] N. Chungsamarnyart, W. Jansawan, T. Tassanawat, T. Thiapayarak, Efficacy of herbal Acaricide; K U Natural Extocide against the Tick, Flea, Lice of dogs. 28th World Congress of the World small animal veterinary Association, October 24-27, 2003, Bangkok, Thailand.

[18] A. A. Adedapo, F. O. Jimoh, S. Koduru, J. A. Afolayan, P. Masika, Antibacterial and antioxidant properties of the methanol extracts of leaves and stems of *Calpurnia aurea*. *BMC Complement. Altern. Med*. 2008, 8 (53), 1–8.

[19] E. M. Varoni, M. Iriti, L. Rimondini, Plant Products for Innovative Biomaterials in Dentistry. *Coatings*, 2012, 2(3), 179-194.

[20] A. Notten, *Gnidia squarrosa* L. (Druce): Kirstenbosch National Botanical Garden http://www.plantzafrica.com/plantefg/gnidiasquar .htm, 2004, accessed on 01/05/2017.

[21] G. Fouche, E. Khorombi, V. J. Maharaj, PCT, Treatment of erectile dysfunction and libido enhancement, US8,609,151 B2, 2013.

[22] G. Fouche, M. Ramafuthulaa, V. Maselelaa, M. Mokoena, J. Senabe, T. Leboho, B. M. Sakong, O. T. Adenubi, J. N. Eloff, K. W. Wellington, Acaricidal activity of the organic extracts of thirteen South African plants against *Rhipicephalus (Boophilus*) *decoloratus* (Acari: Ixodidae). *Vet. Parasitol*.2016, 224, 39-43.