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In vivo and *in vitro* Activities of Medicinal Plants on Haemic and Humoral Trypanosomes: A Review

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Abstract: Reports on the *in vivo* and *in vitro* activities of medicinal plants on haemic and humoral trypanosomes showed that several medicinal plants, worldwide, possessed trypanocidal or trypanostatic activity. The choice of specific plants by researchers were based on their trypanocidal claims as documented in ancient pharmacopoeia, knowledge from traditional healers, herdsmen, village elders and feeding habits of large primates. The plants were subjected to various methods of extraction. The choice of extraction method depended largely on the part of the plan to be tested and often, fractionated through thin layer chromatography, infrared spectroscopy, mass spectroscopy, nuclear magnetic resonance spectroscopy to yield bioactive components. This was with a view of elucidating structural components and possible synthesis of new trypanocides. The commonly encountered active principles in the extracts were saponins, terepins, phenolics, flavonoids, tannins, glycosides, anthraquinones, columbins, neolignan, quinines, phlobatanin, resins and alkaloids. These fractions, produced efficacy ether singly or synergistically at dosages (<800 mg kg^{-1}) *in vivo*, leading to the elimination of parasitaemia, modulating declined red cell indices and the alleviation of clinical signs of trypanosomosis. Most of the extracts however, produced effect *in vitro* within minutes of application in a graded dose manner. The extracts in most cases produced signs of acute toxicity (*in vivo*) at dosages (>800 mg kg^{-1}) leading to degenerative changes in vital organs. Signs of cytotoxicity were also encountered *in vitro* on various cell lines. Therefore, the folkloric medicinal applications of plants for the treatment of trypanosomosis have a pharmacological basis. This may therefore, lead to the synthesis of new, cheap and easily available trypanocides of less toxicity.

Key words: *In vivo*, *in vitro*, medicinal plants, trypanosomes

INTRODUCTION

Over 250, 000 undiscovered flowering plants with medicinal properties exist worldwide (Madureira, 2008). In spite of a rapidly expanding literature on phytochemistry, only a small percentage of the total plant species have been examined chemically and it is still a vast field for research (Gyang, 2001). Several medicinal plants are the most ancient source for the treatment of human and animal trypanosomosis. The use of decoctions from medicinal plants for the treatment of trypanosomosis dates as far back as ancient Egypt, Greece, Mediterranean, India, Assyria and China (Trease and Evans, 1989). Indeed, the discoveries of medicinal plants for the treatment of trypanosomosis have been associated with the study of traditional pharmacopia, wisdom from village elders and traditional healers (Onuaguluchi, 1966; Nwude and Ibrahim, 1980; Aliu and Nwude, 1982; Ibrahim *et al*., 1983). Similarly, the natural instinct and progression of wild primates to utilize medicinal plants in the wild, have often led to the discovery of medicinal plants with antitrypanosomal efficacy (Clayton and Wolf, 1993).

However, in spite of the possible role of medicinal plants as trypanocides (Asuzu and Chineme, 1990; Nok, 2002; Mbaya *et al*., 2007, 2009a, 2010), some of the secondary metabolites in the extracts are toxic in nature (Mbaya *et al*., 2007). Meanwhile, the trypanocidal and trypanostatic efficacy of plant extracts are associated with the presence of one or more biologically active principles (Atawodi *et al*., 2002; Nok, 2002; Mbaya *et al*., 2007). Phytochemical assays have also shown that the antitrypanosomal activity is due to minor components or

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synergistic interaction of all, or some of the active components. Many advances in the field of ethnopharmacology in general, led the World Health Organization (WHO) to develop an international programme, which reviews available scientific data relating to the efficacy of medicinal plants for synthesizing forms that are more effective. This can however, be possible through extraction, separation, isolation, characterization, investigation into the biosynthetic pathways, quantitative evolutions and elucidation of structural formulae of the active ingredients through infra-red spectroscopy, mass spectroscopy, nuclear magnetic resonance spectroscopy (Sofowara, 1982). This may lead to the development of cheap, less toxic and available trypanocides, which will eliminate the problem of drug resistance and relapse (Soerjato, 1996; Cragg *et al*., 1997; Mbaya *et al*., 2009a). In spite of the fact that several plants with trypanocidal efficacy exists, no new, safe and reliable trypanocides have been introduced for the past thirty years, this review was therefore, undertaken to collate reports on *in vivo* and *in vitro* activities of medicinal plants on haemic and humoral trypanosomes as basis for future manufacturing of new trypanocidal drugs.

Historical perspective: Historically, man did not require the modern methods of investigation to collect a material medica of plants, which he often used in conjunction with magical and other ritual practices for the treatment of trypanosomosis. The use of folk medicine is based on knowledge of treatments of ailments based on traditional beliefs, which are common to a group of rural people (Sofowara, 1982). Before the 18th century, only slow progress was made in phytochemistry where compounds of cane sugar, starch, camphor and benzoic acid were virtually used for the treatment of all forms of diseases as a result, hundreds of plants were burnt to yield ashes, which were soaked and used for various therapies (Gyang, 2001). This method, however, led to several disappointments to earlier researchers. In the 19th century, progress became more rapid and in 1903, alkaloids were isolated (Trease and Evans, 1989). This active ingredient among others was found to be trypanocidal (Grand, 1989; Atawodi *et al*., 2003; Abubakar *et al*., 2005; Mbaya *et al*., 2007). In the 20th century, several plants with trypanocidal activity were discovered worldwide (Igweh and Onabanjo, 1989; Sepulveda-Boza and Cassele, 1996; Freiburghaus *et al*., 1996, 1997, 1998, Muellas-Serrano *et al*., 2000; Weniger *et al*., 2001; Nok, 2002; Mbaya *et al*., 2007, 2009a; 2010). However, in South America's aboriginal societies, ethnobotanists are currently fighting a battle against time to record such information before such vital knowledge is lost (Trease and Evans, 1989).

Methods of extraction of trypanocidal plants: The extraction of bioactive agents from plant materials is one of the most intensive of natural products research today and yet the field is far from being exhausted (Gyang, 2001). The choice of a specific method or solvent depends largely on the nature of the plant material and the component to be isolated. Dried materials were often pulverized into fine particles before exhaustive extraction, whereas, fresh leaves and succulent portions were homogenized and extracted using a suitable solvent (Mittal *et al*., 1981; Sofowara, 1982; Gyang, 2001).

In most situations, where air dried materials were powdered into small particles, extraction was most productive with 100% methanol or ethanol (Kaltungo, 1977; Rabo *et al*., 2000; Fabiola *et al*., 2002; Atawodi *et al*., 2003; Samia *et al*., 2006; Mbaya *et al*., 2007; Mikail, 2009). In other cases, ethanol and water were used (Fabiola *et al*., 2002; Kamanzi *et al*., 2004; Wurochekke and Nok, 2004; Wurochekke *et al*., 2004; Sara *et al*., 2004; Ndjakou *et al*., 2007; Shuaibu *et al*., 2008; Ogbunugafor *et al*., 2008; Nibret *et al*., 2009). In situations where succulent leaves such as aloe vera or fruits were extracted to obtain pulp (Nok *et al*., 1996; Abubakar *et al*., 2005), small pieces were obtained, before extraction. When seeds were involved, separation from the pulp using a wire mesh was necessary. Chloroform was used as solvent in some cases (Samia *et al*., 2006). In aqueous extraction, water was used until a good yield (v/v) was obtained (Rabo *et al*., 2000; Igweh *et al*., 2002; Patricia *et al*., 2005; Nwodo *et al*., 2007).

***In vivo* and *in vitro* toxicity of crude plant extracts:** Although, immense traditional knowledge exists in the ethnopharmacology of trypanosomosis, accidental poisonings due to over dosages have been reported (Daziel, 1973; Nwude and Ibrahim, 1980; Rabo, *et al*., 2000). Hence, scientific evaluation of toxicities by determining lethal dosages (LD_{50}) or lethal concentrations (LC_{50}) usually preceded *in vivo* trypanocidal efficacy trials (Mbaya *et al*., 2007, 2009a, 2010). During *in vitro* studies, cytotoxicity in mammalian cell cultures has been documented (Camacho *et al*., 2003; Sara *et al*., 2004; Patricia *et al*., 2005).

***In vivo* toxicity studies:** The toxicity of the decoction from the stem bark of *Butyrospermum paradoxum* (Sapotaceae) sub. sp. Parkii (G. Don) Hepper used in Nrtheastern Nigeria for the treatment of trypanosomosis was evaluated *in vivo* in rabbits (Rabo, 1998; Rabo *et al*.,

2000) and in rats (Mbaya *et al*., 2007). Following the intra-peritoneal administration of the methanolic extract of the stem bark, doses (>80 mg kg^{-1}) produced behavioural changes, morbidity and mortality in the rodents. The symptoms, which were dose dependent, included anorexia, dehydration, depression, prostration, coma and death and at necropsy, congestion with oedema of the lungs, bronchi, bronchioles, kidneys, hepatomegally, with focal necrosis of hepatocytes. Similarly, Mbaya *et al*. (2009a) observed similar but transient signs of toxicity in rats administered derivatives of *Artemisia annua*. The root extract of *Mitragyna ciliata* at dosages (>800 mg kg^{-1}) produced acute signs of toxity in mice (Ogbunugafor *et al*., 2008). Signs of toxicity for most extracts were observed generally above 800 mg kg^{-1} (Rabo, 1998; Rabo *et al*., 2000; Mbaya *et al*., 2007; Ogbunugafor *et al*., 2008). On the other hand, dosages (<800 mg kg^{-1}) of *Annona senegalensis* Pers. leaf did not lead to fatality in mice (Ogbadoyi *et al*., 2007). Similarly, the crude extracts or dihydrochelerythrine derivatives from *Garcina lucida* produced little toxicity *in vivo* (Jean *et al*., 2007).

Cytotoxicity (*in vitro*) studies: An *in vitro* evaluation of the efficacy of *Holarrhena africana* fractions on *Trypanosoma brucei rhodesiense*, showed that one fraction designated as HaF (5) showed no overt cytotoxicity against L-6 cells (Nwodo *et al*., 2007). Meanwhile, evaluation of cytotoxicity of trypanocidal Beninese plants showed that *Hymenocardia acida*, *Trichilia emetica* leaves, were cytotoxic to mammalian cells at higher IC_{50}'S but with the exception of methylene chloride leaf extract of *Strychnos spinosa* (Sara *et al*., 2004). Ndjakou *et al*. (2007) evaluated the cytotoxic effects of some selected Cameroonian plants with efficacy against *T. cruzi* and *T. b. rhodesiense*. Cytotoxicity and selectivity index (SI (b) = 22.5) was higher with the methanolic stem bark extract of *Albizia zygia*. Meanwhile, methylene extracts of *Anogeissus leiocarpus* and *Terminalia avicennoides* on fibroblast did not reveal serious toxicity at moderate concentrations but was toxic to the cells at higher concentrations (Shuaibu *et al*., 2008).

It was also observed by Patricia *et al*. (2005), that extracts of Brazilian medicinal plants with trypanocidal activities, such as *Bacharis trimera*, *Cymbopogon citratus*, *Matricaria chamomilla*, *Mikania glomerata*, *Ocimum gratissimum*, *Piper regnellii*, *Prunus domestica*, *Psidium guajava*, *Sambucus canadensis*, *Stryphnodendron adstringens*, *Tanacetum parthenium and Tanacetum vulgare* showed no toxic effect on sheep erythrocytes, *in vitro*. A methanolic and aqueous extraction of 43 plant species, selected from ethnopharmacological and chemical taxonomic data with possible antitrypanosomal properties showed efficacy against *T. brucei*, however, *Annona purpurea* was the most toxic to KB cells (Camacho *et al*., 2003).

Phytochemical screening of various trypanocidal plants used in the folkloric treatment of trypanosomosis: Earlier workers (Bisset and Phillipson, 1971; Kerharo and Adam, 1974; Oguakwa *et al*., 1980; Ohiri *et al*., 1983; Grand, 1989; Nok *et al*., 1996; Rabo, 1998) isolated active components from plant materials used in the treatment of trypanosomosis. In recent years, workers (Ohiri *et al*., 1983; Copp *et al*., 2003; Atawodi *et al*., 2003; Sara *et al*., 2004; Patricia *et al*., 2005; Nok *et al*., 2005; Abubakar *et al*., 2005; Jean *et al*., 2007; Nwodo *et al*., 2007; Ogbadoyi *et al*., 2007; Ogbunugafor *et al*., 2008; Shuaibu *et al*., 2008; Nyasse *et al*., 2004; Nibret *et al*., 2009; Mbaya *et al*., 2007 2009a, 2010) have isolated various compounds with trypanocidal activities.

The immense chemical constituents and range of biodiversity of plants may in the future, lead to the development of hundreds of pharmacological agents with trypanocidal activities. The active components in the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides* were hydrolysable tannins (Shuaibu *et al*., 2008). Most of the Nigerian Savannah plants such as *Khaya senegalensis, Piliostigma reticulatum, Securidaca longependuncula*ta and *Terminalia avicennoides* contain mainly alkaloids, flavonoids, phenolics and terepins (Grand, 1989; Atawodi *et al*., 2003). Grand (1989) also encountered similar biological components in the leaves of *Piliostigma reticulatum*. Nok *et al*. (1996), reported that *Allium sativum* (Liliaeceae) produced four fractions; ethyl acetate/methanol, ethyl acetate/ethanol, benzene/ methanol and acetic acid/methanol. Among these fractions, the acetic acid/methanol fraction retained the trypanocidal feature of the crude extract. Crude methanolic and dichloromethane extracts from the flowers of *Solanecio angulatus* yielded alkaloids (Nibret *et al*., 2009). The authors also observed that the dichloromethane extract of *Crotalaria phillipsiae* twigs yielded Senecionine. Nok *et al*. (2005) demonstrated that the plant *Aristolochia albidia* yielded dipterpenoid furanolactone (columbin), a potent trypanocide, while Ogbadoyi *et al*. (2007) showed that *Annona senegalensis* leaf extract contained mainly tannins, phlobatanins and saponins. Similarly, the ethanolic extract of the stem bark of *Butyrospermum paradoxum* (Sapoataceae) was found to contain tannins and alkaloids (Rabo, 1998; Mbaya *et al*., 2007).

One fraction designated as HaF (5) was obtained from the aqueous extract of young leaves of *Holarrhena africana*, a plant used in Nigerian traditional medicine (Nwodo *et al*., 2007). Mbaya *et al.* (2010) also showed that the ethanolic extract of the stem bark of *Azadirachta indica* contained salanin, melzantriol, nimbin, cardiac glycosides, tannins, alkaloids and saponins produced a remarkable trypanocidal activity *in vivo* and *in vitro*. Similarly, Abe *et al.* (2002) isolated Eupomatenoid-7 (neolignan) and fragransin (lignan) from crude leaves and flower extract of *Aristolochia taliscana*, a potent anti *T. cruzi* derivative. In a bid to evaluate the phytochemical components of the fresh pulp of some trypanocidal Nigerian plants, *Aloe vera* showed heavy presence of tannins, resins and alkaloids (Abubakar *et al*., 2005). In the same study, *Mamordica balsamina* had more of glycosides while *Annona senegalensis* leaves had more of tannins followed by glycosides and less of flavonoids and saponins (Abubakar *et al*., 2005). Similarly, the authors also reported that *Securidaca longipendunculata* root and root barks had high concentrations of alkaloids, flavonoids and saponins.

Three benzo [c] phenanthridine alkaloids were isolated from the stem bark of *Garcinia lucida* and proven to be trypanocidal at the same time, its new derivative , (S) 1-(9,10-dihydro-7, 8-dimethoxy-10-methyl-1-1, 2-benzophen anthridine-9-yl) propan-2-one (lucidamine A) (S) was produced semi synthetically. The crude extract as well as the synthetic derivatives produced excellent antitrypanosomal activity (Jean *et al*., 2007). Sara *et al.* (2004) analysed the crude methylene chloride leaf extracts from potential trypanocidal plants from Benin such as *Hymenocardia acida, Strychnos spinosa, Cassia sieberiana* and *Trichilia emetica*. Tannins, flavonoids and quinones were the active principles encountered. Tannins have equally been identified decades ago in the leaves of all species of *S. spinosa* (Watt and Breyer-Brandwijk, 1962; Persinos and Quimby, 1967; Doquenois and Anton, 1968; Kerharo and Adam, 1974).

A literature survey indicated that several flavonoids have antitrypanosomal activity (Raz, 1998; Camacho *et al*., 2000; Tarus *et al*., 2002). *C. sieberiana* have been shown to contain anthracenic derivatives (Doquenois and Anton, 1968; Nok, 2002). Ogbunugafor *et al.* (2008) during a chemical analysis of the active fraction of *Mitragyna ciliata* and *Pelleger* (Rubiaceae) showed that, ethanolic root extracts of the plants yielded alkaloids.

***In vivo* effect of medicinal plants on humoral trypanosomes:** Table 1 shows the various medicinal plants reported to have *in vivo* anti trypanosomal activity against humoral trypanosomes. *Trypanosoma brucei* group of trypanosomes such as *T. brucei brucei*, *T. evansi, T. b. rhodesiense* and *T. brucei gambiense* were classified as humoral (Losos and Ikede, 1972; Mbaya *et al*., 2009b). For *T. cruzi*, however, it exists in two forms, trypamastigote in the blood (haemic) and amastigote (humoral) intracellularly in the tissues (Losos and Ikede, 1972). However, *T. cruzi* along with the *T. brucei* group are humoral due to their preference for solid tissues, particularly in loose connective tissue stroma and fluids of body cavities. They are able to elicit both humoral and cell mediated immune response. In the extra vascular sites, the organisms elicit cellular infiltrations and degenerative changes. *Morinda lucida* was reported to posses a remarkable effect trypano-suppressive property on *T. brucei in vivo* (Asuzu and

Table 1: Plants with *in vivo* antitrypanosomal effects against humoral trypanosomes

Parts tested	Types of medicinal plants	Trypanosomes	References
Root	*Securidaca longependuuculata*	*T. b. brucei*	Aderbauer *et al.* (2008a)
Leaves	*Guiera senegalensis*		
Flowers	*Solanecio angulatns*		Nibret *et al.* (2009)
Twigs	*Crotalaria phillipsiae*		
Leaf	*Holarrhena africana*		Nwodo *et al.* (2007)
Stem bark	*Garcinia lucida*		Jean *et al.* (2007)
Pulp	*Mamordica balsamina*		Abubakar *et al.* (2005)
	Aloe vera		
Root/bark	*Securidaca longipenduuculata*		
Leaves	*Annona senegalensis*		Ogbadoyi *et al.* (2007)
Leaves	*Aristolochia bacteolata*	*T. evansi*	Samia *et al.* (2006)
Pulp	*Allium sativum*	*T.b. brucei*	Nok *et al.* (1996)
Whole root	*Annona senegalensis*		Ogbadoyi *et al.* (2007)
Root	*Mitragyna ciliata*		Ogbunugafor *et al.* (2008)
Root	*Lawsonia inermis*		Atawodi *et al.* (2003) and Wurochekke *et al.* (2004)
Root	*Crassonia zimmermanni*	*T. b. rhodesiense, T. cruzi*	Martin *et al.* (2007)
Stem bark	*Butryrospermum paradoxum*		Mbaya *et al.* (2007)
Stem bark	*Artemisia annua*		Mbaya *et al.* (2009a)
Leaf	*Morinda lucida*		Asuzu and Chineme (1990)
Leaf	*Aristolochia albida*		Nok *et al.* (2005)
Stem bark	*Azadirachta indica*		Mbaya *et al.* (2010)

Chineme, 1990). Root extract of *Securidaca longependuncula* (*Polygalacea*), leaf extract of *Guiera senegalensis* (Combretaceae) (Aderbauer *et al*., 2008a), flowers of *Solanecio angulatus* and twigs of *Crotalaria phillipsiae* (Nibret *et al*., 2009) showed moderate antitrypanosomal activity against *T. brucei in vivo*. Similarly, Nwodo *et al*. (2007) reported that aqueous young leaf extract of *Holarrhena africana* caused complete disappearance of *T. brucei in vivo*, without relapse. Jean *et al*. (2007) also, showed that extract of stem bark of *Garcinia lucida* displayed attractive activity against *T. brucei in vivo*. The pulp of several Nigerian plants such as *Mamordica balsamina*, *Aloe vera* and *Annona senegalensis* leaves prolonged the survival period of *T. brucei* infected rats (Abubakar *et al*., 2005).

The therapeutic effect of *Aristolachia bacteolata* showed remarkable activity *in vivo* against *T. evansi* (Samia *et al*., 2006). An *in vivo* analysis of the diterpenoid furanolactone (columbin) from *Aristolachia albida* in *T. brucei* infected mice revealed that 25 mg kg^{-1} administered for three consecutive days, cleared the parasites from circulation (Nok *et al*., 2005). The only set back according to the authors, was that columbin could not clear parasites in the cerebrospinal fluid. Nok *et al*. (1996) showed that the oily extracts of *Allium sativum* (*Liliacea*) at one point it completely suppressed the ability of *T. b. brucei* to infect mice and at another, it cured mice in 4 days at 120 mg/kg/day. After fractionization by column chromatography, the acetic acid/methanol fraction retained trypanocidal features of the crude extract. The authors reported that the extract-contained diallyl-disulfide (DAD) which, interfered with the parasites ability to synthesize membrane lipids. Ogbadoyi *et al*. (2007) evaluated the chemotherapeutic effects of crude and partially purified aqueous extracts of leaves, whole root and stem bark of *Annona senegalensis*. The extracts at a dosage of 200 mg/kg/day completely cured mice of *T. brucei*. Ogbunugafor *et al*. (2008) showed that butanolic root extract of *Mitragyna ciliata* (Rubiaceae) possessed trypanocidal activity. They observed a correlation between calcium concentration and parasitaemia, suggesting that the active agent had effect on the calcium metabolism in the rodents, which was deleterious to the organism. Wurochekke *et al*. (2004) reported that the crude methanolic extract of *Lawsonia inermis* had trypanocidal effect against *T. brucei in vivo*. A concentration of 8.3 mg mL^{-1} ameliorated clinical signs but did not affect the level of parasitaemia and packed cell volume in mice even when an adjuvant (glycerol) was added. The crude methanolic extract of the plant *Butyrospermum paradoxum* (*Sapotacea*) found in the Nigerian savannah, produced a remarkable trypanocidal effect, through complete suppression or delay in parasite stablishment with a reduction in the level of parasitaemia and severity of clinical signs as well as enhanced the survival of rats infected with *T. brucei* (Rabo, 1998; Mbaya *et al*., 2007). Similarly, Nok *et al*. (1993) and Mbaya *et al*. (2010) showed that *Azadirachta indica* possesses remarkable trypanocidal effect with a reduction in the level of *T. brucei* parasitaemia *in vitro* and *in vivo* respectively.

***In vitro* effect of medicinal plants on humoral trypanosomes:** Table 2 shows various medicinal plants with *in vitro* antitrypanosomal activity against humoral trypanosomes. In this method, trypanosomes were propagated in specialized media assayed with crude extracts of medicinal plant materials at various concentrations (Nok *et al*., 1993; Atawodi *et al*., 2002, 2003; Igweh *et al*., 2002; Mbaya *et al*., 2010). This was followed by incubation at 37°C and parasitaemia determination (Kamanzi *et al*., 2004; Wurochekke and Nok, 2004; Patricia *et al*., 2005; Nwodo *et al*., 2007; Ndjakou *et al*., 2007; Shuaibu *et al*., 2008; Aderbauer *et al*., 2008b; Mikail, 2009).

The methanolic extracts of various parts of the plants; *Securidaca longependuncula*, *Khaya senegalensis*, *Piliostigma reticulatum* and *Terminalia avicennoides* harvested from the Savannah vegetation belt of Nigeria, exhibited strong trypanocidal activity against *T. brucei* while *Lawsoni inermis* roots, *Prosopis africana* and *Sterculia setigera* slightly reduced motility *in vitro* (Atawodi *et al*., 2003). Similarly, an *in vitro* trypanicidal activity of 13 medicinal plants used by local herdsmen in Northern Nigeria for the treatment of trypanosomosis, showed that the aqueous root bark extract of *Khaya senegalensis* had the highest activity, *Tamarindus indica* was less effective while the stem bark of *Albizia lebbeck* was not (Wurochekke and Nok, 2004).

Seven selected Cameroonian medicinal plants, traditionally used to treat malaria, showed that the methanolic extract of *Albizia zygia* (Fabaceae) stem bark was effective against *T. b. rhodesiense* and *T. cruzi* (Ndjakou *et al*., 2007). Meanwhile, in Côte d' Ivoire, the *in vitro* antitrypanosomal activity of crude ethanolic extract of 101 medicinal plants in that region, showed that *T. b. rhodesiense* was most sensitive to *Enantia polycarpa* (Annonaceae) and *Trichilia emetica* (Meliaceae) (Kamanzi *et al*., 2004). Similarly, the trypanocidal activity of petroleum ether extracts of the root bark of a Tanzanian medicinal plant; *Cussonia zimmermanii* was found to be effective against *T. b. rhodesiense* and *T. cruzi* (Martin *et al*., 2007). The antitrypanosomal activity of the methanolic extracts of

Table 2: Plants with *in vitro* antitrypanosomal effects against humoral trypanosomes

Part tested	Types of medicinal plants	Trypanosomes	References
Roots, stem bark	*Securidaca longependunculata*	*T.b. brucei*	Atawodi *et al.* (2003)
Flowers	*Khaya senegalensis*		
	Piliostigma reticulatum		
	Terminalia avicennoides		
	Prosopis africana		
	Sterculia setigera		
Roots	*Khaya senegalensis*		Wurochekke and Nok (2004)
	Tamirandus indica		
Stem bark	*Albizia zygia*	*T. b. rhodesiense*	Ndjakou *et al.* (2007)
	T. cruzi		
Stem bark	*Enantia polycarpa*	*T. b. rhodesiense*	Kamanzi *et al.* (2004)
	Trichilia emetica		
Stem bark	*Anogeissns leiocarpus*	*T.b. brucei*	Shuaiba *et al.* (2008)
	Terminalia avicennoides		
Stem bark	*Khaya senegalensis*		Mikail (2009)
	Sclerocarya birrea		
	Commiphora kerstngii		
Young leaves	*Holarrhena africana*	*T. b. rhodesiense*	Nwodo *et al.* (2007)
Stem bark	*Azadirachta indica*	*T. b. brucei*	Mbaya *et al.* (2010)
Root bark	*Securidaca longependunculata*	*T.b. brucei*	Aderbauer *et al.* (2008a)
Leaves	*Guiera senegaleusis*		
Stem bark	*Bacharis trimera*	*T. cruzi*	Patricia *et al.* (2005)
	Cymbopogon citratus		
	Matricaria chamomilla		
	Mikaria glomerata		
	Ocimum gratissimum		
	Piper regnellii		
	Prunus domestica		
	Psidium guajava		
	Sambucns canadensis		
	Stryphnodendron adstringens		
	Tanacetum parthenium		
Flowers	*Selanecio angulatns*	*T.b. brucei*	Nibret *et al.* (2009)
Twigs	*Crotalaria phillipsiae*		
Stem bark	*Aristolochia albidia*		Nok *et al.* (2005)
Stem bark	*Ocimum gratissimum*	*T. cruzi*	Fabiola *et al.* (2002)
	Lippia alba		
	Piper regnellii		
	Stryphnodendron adstringens		
	Tanacetum vulgare		
	Psidium guajava		
	Psidium guajava		
	Punica granatum		
Stem bark	*Lawsonia inermis*	*T.b. brucei*	Wurochekke *et al.* (2004)
Stem bark	*Mitragyna ciliata*		Ogbunugafor *et al.* (2008)
Leaves, twigs	*Cassia sieberiana*		Sara *et al.* (2004)
Leaves	*Hymenocardia acida*	*T. b. rhodesianse*	
	Pericopsis laxiflora		
	Trichilia emetica		
	Strychnos spinosa		
Leaves	*Brassica oleracea*	*T.b. brucei*	Igweh *et al.* (2002)
Flowers	*Solanecio* sp.		
Flowers, stem,	*Enantia polycarpa*	*T. b. rhodesianse*	Atendehou *et al.* (2004)
Root	*Trichilia emetica*		
Flowers, stem	*Annona purpurea*	*T. b. brucei*	Camacho *et al.* (2003)
	Alstonia macrophylla		

Anogeissus leiocarpus and *Terminalia avicennoides* were evaluated *in vitro* against *T. brucei* among other trypanosomes. The extracts were found to be effective with Minimum Inhibitory Concentration (MIC) value range of 12.5-50 mg mL^{-1} (Shuaibu *et al*., 2008). Following earlier reports on the trypanocidal activity of *Khaya senegalensis* Atawodi *et al.* (2003), Wurochekke and Nok, 2004) and Mikail (2009) demonstrated similar activity with *Khaya senegalensis* among others (*Sclerocarya birrea* and *Commiphora kerstingii*) against *T. brucei in vitro* at concentrations of 2 and 4 mg mL^{-1}, respectively. The aqueous young leaf extract of *Holarrhena africana*, a plant used in the Nigerian traditional medicine system, exhibited a good activity against *T. brucei in vitro*. On fraction designated as HaF (5) showed an *in vitro* activity against *T. b. rhodesiense* (Nwodo *et al*., 2007).

In Mali and Burkina Faso, trypanocidal effects of lipophilic extracts of medicinal plants showed that the root bark of *Securidaca longependunculata* (Polygalaceae) and the leaf extract of *Guiera senegalensis* (Combretaceae) reduced parasitaemia *in vitro* (Aderbauer *et al*., 2008a, b). In Brazil, extracts obtained from 19 species of plants, used traditionally for the treatment of various ailments, were tested against epimastigote forms of *T. cruzi in vitro*. The results showed that *Bacharis trimera*, *Cymbopogon citratus*, *Matricaria chamomilla*, *Mikania glomerata*, *Ocimum gratissimum*, *Piper regnellii, Prunus domestica, Psidium guajava, Sambucus canadensis, Stryphnodendron adstringens, Tanacetum parthenium* and *Tanacetum vulgare* had significant effect against *T. cruzi in vitro* (Patricia *et al*., 2005).

The *in vitro* effects of crude methanol and dichloromethane extracts of 19 Ethiopian plants and 4 pure pyrrolizidine alkaloids on *T. brucei* was evaluated (Nibret *et al*., 2009). The most active extract was the dichloromethane extract of *Solanecio angulatus* flowers, where the reduced alkaloid extract prepared from *S. angulatus* flowers followed by an acid base extraction, showed more antitrypanosomal activity than the unreduced alkaloid extract. The authors also reported that the second most active extract was the dichloromethane extract of *Crotalaria phillipsiae* twigs while others, showed moderate activity. The diterpenoid furanolactone (columbin) isolated from *Aristolochia albida* inhibited culture forms of *T. brucei* (Nok *et al*., 2005). *in vitro* analysis of columbin at 5-250 μg mL^{-1} showed complete lysis of the parasite within 10-20 min post-incubation.

In Maringá, Parana, Brazil, the efficacy of crude extracts or essential oils of 15 medicinal plants such as *Ocimum gratissimum, Lippia alba, Piper regnellii, Stryphnodendron adstringens and Tanacetum vulgare* showed severe anti trypanosomal activity (Fabiola *et al*., 2002). However, they observed, that *Psidium guajava* and *Punica granatum* produced a lower activity as against *Achillea millefolium, Eugenia uniflora, Mikania glomerata, Plantago major* while *Spilanthes acmella* had no effect on *T. cruzi in vitro*.

The *in vitro* efficacy of the crude methanolic leaf extract of *Lawsonia inermis* against *T. brucei* at a concentration of 48.3 mg mL^{-1} showed that the extract had *in vitro* activity in a graded dose manner (Wurochekke *et al*., 2004). An *in vitro* investigation of the trypanocidal effect on butanolic extract of the root bark of *Mitragyna ciliata* revealed that it had low antioxidative property and the active fraction (alkaloids) may be responsible for its trypanocidal activity (Ogbunugafor *et al*., 2008).

The *in vitro* antitrypanosomal activity of methylene chloride, methanol and aqueous extracts of the leaves and twigs of *Cassia sieberiana* (Caesalpiniacea), *Hymenocardia acida* (Hymenocardiaceae), *Pericopsis laxiflora* (Papilionaceae), *Trichilia emetica* (Meliaceae) and *Strychnos spinosa* (Loganiaceae) used traditionally in Benin for the treatment of human sleeping sickness were evaluated against *T.b. brucei* (Sara *et al*., 2004). The results showed that *Hymenocardia acida* twig and *Strychnos spinosa* leaf and methanolic chloride extracts of *Trichilia emetica* leaf were most active with MIC values <19 μg mL^{-1}. The authors also reported that the determination of the IC_{50} values of the methylene chloride leaf extracts on *T. b. brucei* and *T. b. rhodesiense* on two mammalian cell lines showed that all the extracts possessed some antitrypanosomal activity. Igweh *et al*. (2002) demonstrated that aqueous extract of *Brassica oleracea* effectively immobilized *T. b. brucei* within a 3-hour incubation period, which rendered the organism none infective to mice.

Atindehou *et al*. (2004) also evaluated the activity of 101 crude ethanolic extracts derived from 88 medicinal plants from Côte d' Ivoire through *in vitro* studies using *T. b. rhodesiense*. They observed that extracts from *Enantia polycarpa* (Annonaceae) and *Trichilia emetica* (Meliaceae) were the most promising ones. Their IC_{50} values were 0.5 and 0.04 mg mL^{-1} with selective indexes of 616 and 209 respectively. Camacho *et al*. (2003) observed that the methanolic and aqueous extracts derived from 43 plant species, showed varied *in vitro* activities. They observed that *Annona purpurea* and *Alstonia macrophylla* had IC_{50} values below 10 mg mL^{-1}, which produced a high activity against *T. brucei*.

***In vivo* and *in vitro* effect of medicinal plants on haemic trypanosomes:** Table 3 shows the various medicinal plant reported to have either *in vivo* or *in vitro* antitrypanosomal activity against haemic trypanosomes. *Trypanosoma congolense* and *Trypanosoma vivax* are the haemic trypanosomes, with effects presented mostly in the cardiovascular system (Losos and Ikede, 1972). In acute *T. congolense* and *T. vivax* infections, petechial haemorrhages occur on serosal surfaces, which are related to disseminate intravascular coagulation. The ability of *T. congolense* to sequester in small vessels and capillaries of the brain, heart, skeletal and other tissues often leads to prolonged pre-patent period (Losos and Ikede, 1972; Maxie and Losos, 1977; Mbaya *et al*., 2007).

***In vivo* approach:** The acclaimed *Butyrospermum paradoxum* (Sapotaceae) stem bark and *Azadirachta indica* are commonly used for the treatment of human and

Table 3: Plants with *in vivo* and *in vitro* antitrypanosomal effects against haemic trypanosomes

Part tested status	Types of medicinal plants	Trypanosomes	References
Stem bark	*In vivo Butyrospermum paradoxum*	*T. congolense*	Mbaya *et al.* (2007)
Pulp	*In vivo Allium sativum*	*T. congolense*	Nok *et al.* (1996)
		T. vivax	
Stem bark,	*In vitro Khaya senegalensis*	*T. congolense*	Atawodi *et al.* (2003)
Root bark	*Piliostigma reticulatum*		
	Securidaca longependunculata		
	Terminalia avicennoides		
	Anchomones difformis		
	Cassytha sp.		
	Lancea kerstingii		
	Parkia clappertioniana		
	Strigia sp.		
	Adansonia digitata		
	Prosopis africana		
Stem bark	*Anogeissns leiocarpns*		Shuaiba *et al.* (2008)
	Terminalia avicennoides		

animal trypanosomosis, in northeastern Nigeria. Under scientific trials, the former, produced antitrypanosomal effect by completely preventing the establishment of *T. congolense* infection when administered simultaneously with infection (Mbaya *et al*., 2007) and the later, in *T. brucei* infected rats (Mbaya *et al*., 2010). The extracts produced remarkable antitrypanosomal effects through complete suppression with reduction in the level of parasitaemia and the severity of the attendant disease. Similarly, the oily extract from the pulp of *Allium sativum* (Liliaceae) cured experimental *T. vivax* and *T. congolense* infection in mice at 120 mg/kg/day (Nok *et al*., 1996). They also reported that *Allium sativum* inhibited phospholipidase from the organisms and that column chromatography fractionization produced acetic acid/methanol with trypanocidal feature of the crude extract.

***In vitro* approach:** Atawodi *et al.* (2003) reported that extracts from 23 plants harvested from the Savannah vegetation belt of Nigeria, had trypanocidal activity against *T. congolense* at concentrations of 4, 0.4 and 0.04 mg mL^{-1}. They observed that, extracts of *Khaya senegalensis, Piliostigma reticulatum, Securidaca longependunculata* and *Terminalia avicennoides* were strongly trypanocidal to *T. congolense* within 60 min of application, while, extracts of *Anchomanes difformis, Cassytha* spp, *Lannea kerstingii, Prosopis africana* were trypanocidal to *T. congolense*. The extracts from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides* possessed remarkable trypanocidal activity (Shuaibu *et al*., 2008).

Future prospects: The understanding of the mechanisms of action of chemical compound and their host parasite relationship have led to the development of several new agents with trypanocidal properties (Bacchi *et al*., 1980; Bitonti *et al*., 1986; Fairlamb, 1989). However, drug resistance and relapse parasitaemia are common with all standard trypanocides and in recent years, no new and effective trypanocide have been produced (Onyeyili and Egwu, 1995). Moreover, the associated toxicity, cost and unavailability of the standard trypanocides have been a source of concern. In view of these, ethnopharmacology may lead to the manufacturing of cheap, easily available and less toxic trypanocides (Rabo, 1998; Mbaya *et al*., 2007, 2009a, 2010).

This may be possible when the mechanisms of action of natural products are obtained through isolation, identification and evaluation of bioactive substances (Trease and Evans, 1989). This can therefore, lead to structural modification and synthesis to reduce toxicities, prolong their activity and increase their potency.

CONCLUSIONS

Given the large number (250,000-500, 000) of plant species of which, only 5-15% have been investigated for the presence of bioactive compounds there is need to elaborate an efficient strategy for successful screening. Beside traditional means of flora investigation, ethnomedicine, chemotaxonomy and systemic screening of apes feeding behaviour could be a complementary source of information for targeting plants with trypanocidal properties.

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