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Review

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TETRAPLEURA TETRAPTERA: MOLLUSCICIDAL ACTIVITY AND CHEMICAL CONSTITUENTS

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Abstract

Tetrapleura tetraptera (Schumach. And Thonn) Taub, Mimosaceae, commonly known as Aridan (fruit), A single stemmed, robust, perennial tree of about 30m.It has a grey/brown, smooth/rough bark with glabrous yound branchlets. The flower is yellow/pink and racemes white the fruit has dark brown, four winged pods 12-25 x 3.5-6.5cm.It is generally found in the lowland forest of tropical Africa. The fruit consists of a fleshy pulp with small, brownish-black seeds. The fruit possesses a fragrants, characteristically pungent aromatic odour, which is attributed to its insect repellent property. It is used as spices and aroma (exotic tropical scents) and fish poisoning. It is one of the molluscicidal medicinal plants of Nigeria, also useful in the management of convulsions, leprosy, inflammation and/or rheumatoid pains. The documented biological and-or pharmacological activities are found to be molluscicidal, cardio-vascular, neuromuscular, hypotensive, anti-convulsant, molluscicidal, trypanocidal, hirudinicidal, schistomiasis control, anti-ulcerative, ectoxicity, anti-inflammatory, hypoglycaemic, anti-microbial, emulsifying property, birth control, food value and the control of intestinal parasites. Activity-guided fractionation of the methanol extract of the fruits of *T. Tetraptera* led to the isolation of a saponin glycoside with an oleanolic acid aglycone, a monodesmosidic diglycoside of the rare sapogenin 27-hydroxyolean-12 (13)-en-28-oic acid; echinocystic acid-3-0-sodium sulfate from the stembark, umbelliferone and ferulic acid from the leaves and branches respectively. Also isolated from the fruits were aridanin and three of its olean-12-en-28-oic acid derivatives. All the compounds isolated either from the fruits or other parts were found to exhibit strong molluscicidal properties against the schistosomiasis-transmitting snails *Biomphalaria glabrata.*

Keywords: Molluscicidal,Schistosomiasis, Mimosaceae, *Tetrapleura tetraptera,* aridan, aridanin,

Introduction

There has been an appreciable increase in research on bioactivity of natural products. The biological aspects most researched are antimicrobial, molluscicidal, insecticidal, parasitic, toxicity tests and anti-tumour in decreasing order while advancing a look at African endemic and often neglected diseases such as malaria, leishmaniasis, schistosomiasis, lymphatic filariasis and ochacerciasis, African trypanosomiasis and chargas disease, leprosy, dergue and tubersculosis.

The advent of modern drugs for the treatment of trematode infection has been like a burst of sunshine after a long and particularly dreary night. Where there had been, for half a century, a trickle of drugs of modest efficacy and awesome toxicity, there was the rapid discovery of several excellent medications. Outstanding among them was Praziguantel, which was slightly effective against all major human species of *Schistosomia, Clonorchis, Paragonimus, Opisthorchis, Metaganimus and Fasciolopsis* (Campbell, 1986). The isolation and characterization of natural products from African medicinal plants without any biological or pharmacological testing has yielded

numerous compounds of novel structure and constitutes the majority of all the recent publications on African Medicinal Plants (Sofowora, 1993).

Schistosomiasis transmission in Nigeria has a long history. It was reported that the Fulani's brought schistosomiasis from the Nile in Egypt during the time of their migration to Nigeria. *Schistosomia haematobium* is more common in West Africa than *S. mansoni*, studies of parts of South West Nigeria showed that intestinal schistosomiasis is present that *S. mansoni* was encountered in a dam site in Ile-Ife. It has also been reported that transmission, endemicity and focality of intestinal and urinary schistosomiasis were present in other communities around man-made dam in Osun State of Nigeria (Adewunmi, et al. 1993).

One of the methods for the control of this disease is the use of molluscicides and the urge for the use of plant molluscicides has received increased interest primarily because it could be an appropriate and inexpensive technology for snail control in endemic poor nations of the World. In Eastern parts of Nigeria, the fruits are used to prepare soups for mothers from the first day of delivery. The plant is also used as a mosquito repellant. A purgative and as an emetic. Adewunmi *et al* (1989)

There is still an urgent need for highly potent plant molluscicides in order to avoid the transmission of the parasitic disease schistosomiasis. Maillard, M. et al (1989) and further phytochemical investigation of this and other plants would still be very necessary. *Schistosomiasis* or bilharzia, a disease transmitted by some freshwater snails in Nigeria, most notably of two genera *Biomphalaria* and *Bulinus* is endemic in the country. The infection produces various debilitating effects depending on the person's age, immunological characteristics, the number of worms present and the number of eggs laid. Infections are most severe in children than in adults. The intermediate hosts play an essential role in the schistosome parasitic life cycle. Schistosomal cercarial are released from the snails into the water and penetrate the skin of individuals exposed to the water while engaged in bathing, swimming, fishing and agricultural activities. Molluscicides are therefore very crucial for controlling schistosomiasis if appropriately used. It has been shown that *Aridan* and *aridanin* isolated from *Tetrapleura tetraptera* could interrupt the life cycle of the schistosome by its cercaricidal, miracicidal and molluscicidal properties. The WHO thus recommends that safety testing should parallel developmental stages of a plant molluscicide (Awe et al 1995). The snails belonging to the family Lymnaeidae are known to act as intermediate hosts of both human and animal fascioliasis. The fresh water snail *Lymnaea luteola* Lamarck (Mollusca: Gastropoda), is widely distributed in India and acts as intermediate host of *Schistosomia incognitum, S. nasale, Orlantobilharzia dattae*. *Fasciola hepatica, F. gigantica*, the causative agents of fascioliasis among cattle and cercarial dermatitis in human beings in the Northern part of India. The control of the snails is regarded as one of the best preventive measures in controlling all forms of schistosomiasis. The extracts of few plant molluscicides like *Euphorbia splendens, phytolacca dodecandra, Tetrapleura tetraptera* had been reported to exhibit lower toxicity towards earlier developmental stages than adults. It is also reported that the n-butanol extracts of some plant molluscicides like *Sapindus trifoliatus, Agave americana, Balanites agyptica, Jatrapha gossypifolia, and Vaccaria pyramidata* are toxic against freshly laid eggs of *L. luteola,* while considering safety to non-garget organisms, fishes, and animals, nicotinanilide may be regarded as a better option even though the LC_{90} value is 1.6 times higher than niclosamide (Sukumaran et al., 2004).

181 plant extracts, which represented 106 species in 41 families employed in Nigerian herbal medicine were screened for their molluscicidal activity following the W.H.O. protocol, with laboratory reared snails of known age. Only 23 (12.7%) of these methanol plant extracts, spreading over 106 species in families however, showed 100% mortality to the snails at a concentration of 100 ppm of extract including *T. Tetraptera* and extracts having 100% activity were achieved by the Drug Research and Production Unit of Obafemi Awolowo University (Adewunmi. and Sofowora, 1980). Molluscicіding is still considered the most important means of control of schistosomiasis where the volume of water per caput is small. In rural communities the cost of synthetic molluscicides and/or chemotherapy prohibits their use. Plant molluscicides, applied as crude aqueous suspensions are the source of cheap, effective and environmentally acceptable alternatives. Further, infected communities are likely to accept the use of local indigenous plants, particularly if they have more than one local application, since they are familiar with their properties and growth characteristics. (Clark et al., 1997). In general, health education coupled with the use of chemotherapy and cheap molluscicides are recommended for the control of this disease (Sofowora, 1993).

Despite the success of some control programmes, the prevalence of schistosomiasis remains, largely because population growth and development of man-made water resources is continuing (Lemmich et.al., 1995). Treatment of water bodies with molluscicidal compounds is considered an important element in an integrated strategy for morbidity control, but as the use of synthetic molluscicides is impeded by the high costs, there is a demand for inexpensive alternatives such as natural products.

Schistosomiasis is perhaps second to malaria as a health problem in Africa out of the six TDR (WHO/World Bank sponsored Project on Tropical Diseases Research) diseases. About 141-150 million people on the African continent are infected with this tropical disease caused by waterborne parasites. The disease is transmitted by some fresh water molluscs, notable of the general *Biomphalaria*, *Bulinus* and *Oncomelania.* Schistosomiasis or bilharzias is a disease that affects more than 200 million people in the tropical and sub-tropical areas of the World. The fight against bilharzia is one of the most important challenges in countries of the tropics. The WHO records estimate that about 200 million people are affected by infection with *Schistosonia* species and 400 million more are threatened in at least 76 countries (Bode. et al., 1996).

The chemotherapy of schistosomiasis has been reviewed by Davis (1982) and by Bennett and Depenbusch (1984). The broader topic of the chemotherapy of fluke diseases of man has been reviewed by Campbell and Garcia (1986), while the treatment of intestinal flukes of man has been reviewed by Cross (1985). Aridan (*Tetrapleura tetraptera* Taub., Mimosaceae is one of the molluscicidal medicinal plants of Nigeria (Adewunmi et al. 1988). Comprehensive laboratory evaluation has been conducted on aridan with encouraging results obtained with the aqueous and methanolic extracts of this plant in a schistosomiasis control project conducted at Fasina and Edun Abon (Adewunmi et al., 1982). The plant is therapeutically useful in the management of convulsions, leprosy, inflammation and/or rheumatism pains.

The purpose of this piece is to review the status of important plants in the control of some parasitic diseases of Africa in the continuous efforts towards finding scientific evidence for the claims as to the therapeutic efficacy of African herbs by traditional healers/practitionals.

The interest in studying plant material containing molluscicidal compounds is based on the idea of a local supply of molluscicides, which can be produced at low costs by sinple technologies. The plant *Phytolacca dodecandra* has been a very promising candidate for a plant molluscicide and the development of a safe and affordable molluscicide from the berries of this plant has been achieved. The awareness created by WHO health education programmes in Africa as well as the ease of culturing the intermediate host snails have probably resulted in the increase research activity in the area of plant molluscicides in Africa and elsewhere. Several plants have been screened all over Africa for molluscicidal activity. Three stand out clearly having been well researched up to field trial stages. The first is Endod, *Phytolacca dodecandra*, of which a lot has been published through the work of Lema's group (Lema, 1965; Parkhurst et al., 1989; Lambert et al., 1991). The saponins in *Swartzia madagascariensis* were first reported by Finn Sandberg in 1951 but the plant has been brought again into the limelight by Hostettman's group for the molluscicidal activity of its saponins. The third candidate plant molluscicide from Africa is subject of this review, *Tetrapleura tetraptera* (Aridan) which through the work of Adewunmi (1991) and his collaborations with Swedish, Danish, Italian, Swiss and Nigerian scientists has resulted in the isolation and characterization and testing of new N-acetylglycosides of triterpenoids with molluscicidal activity.

Another new triterpene glycoside, 3- (O-β-D-glucopyranosyp- (1-6) –β-D-glucopyranosyl) – oxy–27–hydroxy-olean—12-en-28-oic acid, was also reported from the fruit of this plant following his collaboration with Hostettman's group in Switzerland (Maillard et al., 1992). A review of African plant molluscicides has also been made by Hostettman (1989).

The Family Mimosaceae

Trees or shrubs, very rarely herbs. Leaves mostly bipinnate, rarely simply pinnate, often with large glands on the rhachis. Flowers hermaphrodite, small, spicate, racemose or capitate, actinomorphic, usually 5-merous. Calyx tubular, valvate or very rarely imbricate, 5-lobed or toothed. Petals valvate, free or connate into a short tube. Stamens equal in number to the sepals or more numerous, free or monadelphous; anthers small, 2-celled, opening lengthwise, often with a deciduous gland at the apex. Ovary superior, of 1 carpel. Fruit mostly dehiscent. Seeds with scanty or no endosperm. Mainly tropics and subtropics, often in dry regions and then frequently spiny; easily recognized by the usually bipinnate leaves and small flowers with valvate petals. Calyx-lobes imbricate.

The Genera Tetrapleura
Tetrapleura tetraptera

The varieties are: *Tetrapleura thonningii and Adenanthera tetraptera(Hutchinson,J.and Dalziel,J.M.).*

Description of *T. tetraptera* and Folkloric Uses

The plant is a single-stemmed, robust, perennial tree with dark green leaves and thick, woody base and spreading branches. The plant has a wide natural distribution over a large part of tropical Africa, especially in the rain forest belt of West, Central and East Africa. The fruit consists of a fleshy pulp with some small, brownish – black seeds. The fruit possesses a fragrant, which has been attributed to its insect repellent property Ojewole et al., (2004). The fruits are green when tender and dark red-brown when fully ripe. The fruits are about 22 cm.-27 cm long,pod 4-5 cm wide and have four longitudinal wing-like rather fleshy ridges about 2 cm. broad of which two are hard and woody. A complete pharmacognostical/botanical description and non-medicinal uses of *Tetrapleura tetraptera* (Schumach and Thonn) Taub. is provided as follows**:** http://www.york:.ac.uk/res/celp/index.htm the bole; small/large 30m, a grey/brown, smooth/rough bark with glabrous yound branchlets. The leaf is bipinnate, 5-7 pinnae, 6-11 pairs of leaflets per pinna, pinnae is opposite/alternate, leaflets is also opposite/alternate; petiole is 4-11 cm; lamina is small 0.5-2x0.5 – 1.5cm oblong/ elliptic, cuneate, emarginated entire, glabrous or hairy beneath, domatia, thorns and spines and glands are absent; flower is yellow/pink and racemes while the fruit has dark brown pods 12-25 x 3.5-6.5cm. It is generally found in the low land forest. The reddish, hard and heavy wood, is used for firewood, building poles, pestles, tool handles and carvings.

The economic and medicinal uses of *T. tetraptera* are many. The fruits are used locally in Nigeria in flavouring, in pomades and in soaps. An infusion of the whole fruit is usually taken by convalescents to bathe in order to get relief from feverish conditions, for use as an enema, for constipation and as an emetic. The soft parts of the fruit and the bark are known to contain sugars, tannins, traces of saponin and amino acids (Adesina et.al., 1980). The plant has many traditional medicinal uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains. Infusion of the whole fruit is taken as a recuperative tonic Ojewole and Adesina (1983).

Phytochemistry

Alcoholic extract of the fruits of *T. tetraptera* possesses molluscicidal activity. Two saponin glycosides were isolated from the extract and their aglycone was found to be oleanolic acid while a compound was isolated from the methanolic extract of leaves and branches and were characterized as umbelliferone and ferulic acid by electrophoresis technique (Adesina et. al. 1980). The Africa Phyto International has listed the fruit to contain essential oils, saponosides triterpenes, -aesculetin, coumarins, scopoletin, tannins, sugars, steroids, triterpene glycosides.

Activity-guided fractionation of the methanol extract of the fruits afforded 4 saponins, which exhibited strong molluscicidal properties against the schistosomiasis-transmitting snails *B. glabrata*. Chemical,enzymatic, and spectral methods (DCI – MS, ^{1}H-NMR, ^{13}C-NMR) showed them to be N-acetylglycosides of oleandic acid and of echinocystic acid (Maillard et. al. 1989).

The nutritional value of fresh mature fruits harvested in Southern Nigeria was assessed and found that the crude protein content was very low in the fleshy mesocarp (2.21%) and seeds (0.51%) while no crude protein was detected in the woody mesocarp. The fruits and seeds were rich in some macro-elements such as potassium, iron, magnesium and phosphorus but sodium content was low. The samples analysed contained <3 mg/100 g of zinc and nickel. The fruits contained the following toxic substances: oxalates (8.14-16.6 mg/100 g), tannins (16.5-35.7 mg/100 g).and hydrocyanic acid (hydrogen cynide) (98-100 mg/100 g). Ascorbic acid content ranged from 25 mg in the seed to 68 mg in the fleshy mesocarp. Sucrose, glucose and fructose were detected in the fruits and seeds. The physical and chemical properties of the oil indicated that it could be regarded as a drying oil with few unsaturated bonds (Dosunmu, 1997).

The L-γ-Methyleneglutamic acid is rather common in higher plants. The homologous L-γ-ethylideneglutamic acid was isolated from *Tulipa gesneriana*. These two amino acids were isolated from the seeds of *Tetrapleura tetraptera* (75.0g), through ion-exchange and paper chromatography and identified by IR, ORD and NMR respectively by Gmelin et al. (1967). In the furtherance of the chemistry of *Tetrapleura tetraptera,* Obidoa et al.(1991), isolated scopoletin as an active principle in the traditional herbal infusion of the fruit of this plant, a recipe in the ethnopharmacology of West Africa. It is a potent hypotensive and non-specific spasmolytic agent. These pharmacological effects of scopoletin are probably the underlying factors in the slowly developing tropical neuropathy characterised by optic atrophy, nerve deafness and ataxia endemic among populations subsisting on

cassava diets such as gari. Hitherto, these toxicities were attributed to cyanogenic glucosides (cyanide) present in cassava.

The nutritional quality of the dry fruit of *T. tetraptera* also was assessed by Essien et al. (1994). The fruit shell, fruit pulp and seed contained varying amounts of nutrients such as protein, lipids and minerals, which were comparable and some were even higher than popular spices such as red pepper, onion, curry and ginger. The crude fibre content of the fruit shell was noteworthy and can be considered a good source of this nutritional factor. The distribution of crude lipids which indicated the location of the aroma of the spice, enhanced the processing of the fruit and improve its use for commercial purposes. Okwu (2003), uniquically reported on the chemical evaluation, nutritional and flavouring, properties of *Tetrapleura tetraptera* in which the spices contain crude protein, (7.44% - 17.50%) crude lipid (4.98% - 20.36%), crude fiber (17% - 20.24%), carbohydrate (43.18% - 49.06%) and food energy (234.42 – 379.48g/cal.). The spices were also sources of minerals e.g. calcium, phospharus, potassium, zinc and iron) while the phytochemical screening revealed the presence of tannins, phenolic compounds, saponins, alkaloids, steroids and flavonoids which could be subsumed to be responsible for its varied biological and pharmacological properties.

Biological/Pharmacological screening for Molluscicidal Activity.
Plants Material

The plant material was collected from Osi-Soko near Ile-Ife and identified by the staff of the Forest Research Institute of Nigeria Herbarium, Ibadan, Nigeria where herbarium specimen was deposited.

Sources of Snails

Bulinus (Phyopsis) globesus were cultivated in laboratory with slight modification of the method of McClelland as described by Adesina et. al. (1980).

Preparation of Extracts and Evaluation of Molluscicidal Activity

The methanolic extract of the fruit was prepared and concentrated *in vacuo* to dryness. A weighed amount of the extract was made up to desired concentrations in water for analysis. The W.H.O. method (II) for testing for molluscicides was followed, exposure and recovery periods were 24 hours in all the tests. The number of replicates usually two and average number of snails per test was 10. When inconsistent results occurred, additional eight replicates were run to ensure a reliable mortality rate. Copper sulphate was used as the reference molluscicide with varying concentrations from 10 to 0.10ppm to monitor the susceptibility of snails and to compare its potency with the extracts while the lethal concentrations and their 95% confidence limits were determined by probit analysis.

The results of the biological evaluation of the molluscicidal properties of *T. tetraptera* showed the mean mortality of snails at different exposure times indicated that the take-up and reaction of snails to the lethal effect of the active principle(s) is both time and concentration dependent, that is the longer the exposure the more sensitive the snails to the molluscicidal action of the extract. The time concentration relationship is to be expected as most of the known molluscicides exhibit this property. Adesina et al. (1980).

The methanolic extract of the fruit killed *Bulinus globorus* in water at a concentration of 1.3ppm in 24 hours. The methanolic extractive was about 11 times as active as the crude ground fruit and with no phytotoxicity. The field evaluation of the fruit methanolic extractive at Fasina and Edun-Abon in the Southwest Nigeria against *B. globorus*, *B. forskahi* and *Lanistes* spp. at a concentration of 10 ppm gave encouraging results confirming what was found in the laboratory tests. This report was a summary of studies conducted primarily in the laboratory, but also on a limited scale in the field on the quantitative evaluation of the molluscicidal properties of Aridan. Adewunmi *et.al*. (1982). The mortality activity was linked to the isolated triterpenoid saponins and coumarins. Adesina et.al. (1980).

It was also demonstrated by Adewunmi et.al. (1982) that there are differences in the susceptibility of the snails to Aridan. *Bulinus globosus* (DRPU strain) was found to be more susceptible to Aridan than other snails such as *Physawaterloti* and *Biomphalaria pfeifferi. B.globosus* (Ghanaian strain) is about 2.4 times as resistant as the Nigerian strain, while the calculated LC_{50} of the methanolic extractive is 1.33 (1.05-2.13). It also indicated that Aridan is very stable when stored over a priod of 15 months, which showed that storage has no significant effect on the activity of Aridan. Adewunmi et al. (1988) also investigated the effect of aridanin in the presence and absence

of calcium chloride and magnesium sulphate because the salt contents of natural waters affect the potency of molluscicides and of serotonin which plays a very important role on molluscan hearts. The results showed that the molluscicide was very potent and has a very stable shelf life. The increased potency of aridanin in the presence of some physico-chemical factors such as calcium and magnesium at a concentration of 8.0-16.0mM showed that the effect of these salts would be an advantage to the molluscicide under field conditions. The dose-dependent increase in the heart rate of *B.glabrata* caused by serotonin was inhibited by aridanin.

The ethanolic extracts of the stembark of *Tetrapleura tetraptera* as shown by El lzzi et al. (1990) exerted an inhibitory effect on the luteinizing hormone (LH) released by cultured rat pituitary cells. The extracted saponins were pinned down to this activity and the results thus explained the anti-gonadotrope properties of the extracts that were used as natural contraceptives in Ivory Coast pharmacopoeia.

On the laboratory and field evaluation of the molluscicidal properties of *T. tetraptera*, Adewunmi et al. (1982) had conducted experiments including the following:

Toxicity to Miracidia and Cercariae

Miracidia and cercariae of *Schistosoma haematobium which* were exposed to various concentrations of Aridan for 2 hours showed that within 30 minutes, a concentration as high as 400ppm has lethal effect on both miracidia and cercariae while concentrations less than 10 ppm showed no effect on their motility.

Adewunmi.et al.(1990) conducted experiments on different stages of *Schistosonia mansoni* and *S. bovis* using various concentrations of aridanin and Aridan. They were found to be active against *S. mansoni* and *S. bovis* miracidia. A low concentrations of aridanin (0.25mg/ml) reduced the production of cercariae by snails already shedding cercariae. Aridanin and Aridan also produced profound reduction in the worm recovery of mice infected with pre-treated cercariae of *S. mansoni* and *S. bovis.* Higher concentrations of the molluscicides were biocidal to the cercariae of these schistosomes which indicated that the molluscicides are capable of reducing the transmission of schistosomiasis at different stages of the schistosome development.

Molluscicidal activity of different parts of *Tetrapleura tetraptera*

The lethal concentrations for the different parts on the snails were compared after exposure to serial dilutions of the methanolic extractive. The fruit methanolic extractive appeared to be the most active part of the plant The results of phytotoxicity studies showed that 10 ppm of the methanolic extractive has no effect on the germination and growth rate of Piscia and tomatoes planted in pots in a garden because there was no significant difference in yield in terms of fruiting capability and dry weight of the plants.

Laboratory and Field Evaluation of Molluscicides

It was found that there was not much difference between the laboratory ppm-hour rated potency of Aridan when compared with Endod and sodium pentachlorophenate. But the field ppm-hour rated potency was about 6 times less as potent as that of sodium pentachlorophenate. The result of the preliminary field evaluation showed that a minimum of 10 ppm of Aridan was required in the field.

Fish toxicity Experiment

Two species of fish, *Tilapia nilotica* and *T. galilaea* were exposed to serial dilutions of Aridan after acclimatisation for two days before experimentation and of varying sizes from 10cm to 15cm in length. Their death were recorded after exposure period of 24 hours. The results showed that LC_{50} for *Tilapia nolitica* and *T. galilaea* were 0.35 and 0.44ppm respectively, an indication that the susceptibility of fish to Aridan varies with species.

Cardiovascular and Neuromuscular Actions of *Tetrapleura tetraptera*

Ojewole and Adesina had in 1983 demonstrated the cardiovascular and neuromuscular actions of the fruit of *T. tetraptera* and were able to pin down the chemical components responsible for such activities as Scopoletin, a coumarin which was isolated from the fruit thus validated some of the folkloric uses of the plant. The

cardiovascular and neuromuscular actions of scopoletin was investigated in some laboratory animals. Pharmacological examination through *in vitro* and *in vivo* experiments showed that it possessed depressor (hypotensive) effects in anaethetized rats, produced negative chronotropic and inotropic responses on guinea-pig isolated atria, inhibited acetylcholine-induced contractures of the toad vectrus abdominis muscle, and depressed or abolished electrically-evoked twitches of the chick isolated biventer-cervicis muscle or rat isolated phrenic-nerve hemidiaphragm muscle preparations. The pharmacological actions of scopoletin on the various organ systems examined were resistant to the actions of specific antagonists such as atropine and physostigmine. This showed that scopoletin possesses hypotensive and neuromuscular-blocking activities.

Ojewole and Adesina (1983) further reported the isolation of scopoletin and the plausible mechanisms of the hypotensive effect of scopoletin through *in vivo* and *in vitro* experiments and thus established that scopoletin produced hypotension in laboratory animals through its smooth muscle relaxant activity by which means it presumably dilates blood vessels and by acting as a non-specific spasmolytic agent like papaverine.

Control of Schistosomiasis and Fascioliasis

Molluscicidal activity of *Tetrapleura tetraptera* was conducted on methanolic extractive of the fruit at a concentration of 7.5 – 60 mg/litre, which caused a slowing of the heart of intact *Biomphelaria glabrata* which was found to be dose and time dependent. Also the water extract of the fruit was applied at a concentration of 15, 20 and 25mg/litre in selected water contact sites in three villages near Ile-Ife, Nigeria over a period of 24 months. Snail surveys were carried out in control and treated locations to assess the effectiveness of the extract. The difference in snail numbers between pre-treatment snail population and post treatment snail population was statistically significant ($P < 0.004$), an indication that the extract was able to control the snails at the treated sites only. It was also found out that *Lymnaea natalensis* was not detected in any of the treated sites (Adewunmi 1984).

It is important that the effect of this plant molluscicide be shown on nontarget organisms before it could be recommended for further development and the chicken happened to be one of these nontarget animals. It was equally shown that *T. tetraptera* had little or no toxic effect on the weight gain and/or blood values of the domestic chicken ($p>0.05$). Blood values analysis for RBC and WBC when compared with exotic breed values recorded in temperate countries raises the need to carry out research into normal blood values of Nigerian domestic chicken and animals. The apparent non-toxic effect of this molluscicide on the domestic flowl warrants further development of the plant as a vegetable molluscicide Olubunmi et al. (1986).

The proof of the efficacy of this plant as a potent molluscicide was further advanced by the histopathological study of *T. tetraptera* extract on some fresh water snails by Adewunmi et al. (1986). The histopathological changes resulting from exposure of *Bulinus* (Phyopsis) *globosus, Biomphalaria glabrata* and *Physa waterlotti* to the methalonic extract of *T. tetraptera* were studied. The effect of the extract on various snail tissues were found to be time and concentration dependent. It was therefore suggested that the epithelium was probably the primary site affected by the molluscicide.

Some Nigerian plants with molluscicidal effects were tested on *B. glabrata* and *Archachatina marginata*. The plants were, *Bridelia atroviridis Tetrapleura tetraptera, Calliandra portoricensis, Rauvolfia caffra* and *Jatropha gossypytolia*. Oruwacin and anthraquinones isolated from *Morinda lucida* were also tested. A considerable differences exist on the responses of the *in vivo* heart of *B. glabrata* and *in vitro* heart of *A. marginata* to some of the plant molluscicides. It described the action of some plant on molluscan heart and explained their effects on the heart of *B. glabrata* (aquatic snail) and *A. marginata* (giant African land snail). The response of the heart of *B. glabrata* to various molluscicides varied from reduction to an increase. The most promising plant was *T. tetraptera*, which fruit showed an LC_{50} on *B. glabrata* of 1.95 (0.98 – 2.32) mg/L while the most promising chemical isolated from *M. lucida* was oruwacin with an LC_{50} of 3.50 (1.94)mg/L . The fruit of *J. gossypytolia* and *T. tetraptera*, the root of *R. caffra C. portoricensis* and *B. atroviridis* were the most active parts of the plants which confirmed an earlier study, Adewunmi (1984). It was equally observed that oruwal, *T. tetraptera, C. portoricensis,* and *J. gossypytolia* caused a reduction in heart rate of *B. glabrata* and *A. marginata* Adewunmi et al. (1986).

Nwaiwu *et al.* (1986) also reported the anticonvulsant activity of the volatile oil from the fresh fruits of *Tetrapleura tetraptera* in mice and the results obtained showed that the oil given intraperitonally offered some protection against leptazol – induced convulsions. A dose of 0.4ml of the oil per mouse protected 78% of the animals whom administered 30 min prior to leptazol. While 0.6ml offered no protection from death, the onset of convulsions and the average time of death was prolonged. The effects of an aqueous fruit extract of *T. tetraptera* was investigated in cats, dogs, rabbits and rats as an upgrade in subjects used. Pharmacological examination showed

that it possessed little or no hypotensive effect in cats, dogs and rabbits, but significantly depressed the blood pressure of anaesthetized rats (Adewunmi et. al., 1987).

The proof of the mechanism of activity of this plant was further demonstrated with comparison to a well known molluscicide, bayluscide. The effect of chronic application of sublethal concentrations of aridanin (0.25 – 0.125 ppm) and of bayluscide (0.05 – 0.025 ppm.) on the glycogen and protein content of *Biomphalaria glabrata* was determined and was found that aridanin and bayluscide produced significant reductions in the glycogen content of *B.glabrata*, but a significant decrease in the protein content of the snails was not apparent until after 4 weeks of continuous exposure. The results however indicated that the molluscicides may exert their primary molluscicidal action on the carbohydrate metabolism of the snail (Adewunmi et al., 1988).

As a part of studies to assess the safety of the isolated molluscicidal compound aridanin, a whole-body distribution study of the radioactivity labelled compound was performed in mice. The distribution of ^{3}H-Aridanin was also studied in the target snail, *B.glabrata* to possibly identified specific uptake which might be connected to the toxicity of the compound. The molluscicide aridanin which was labelled with ^{3}H and its tissue distribution was studied in mice and snails by whole-body autobiography and liquid scintillation. In mice intravenously injected 3H-Aridanin was rapidly taken up in the kidney and liver. Its elimination-mainly through the faeces and to a lesser extent through the urine was rapid. About 40% of the total aridanin dose was excreted after 24 hours. No specific retention in any tissue was observed after 24h and Aridanin did not pass the placental barrier in pregnant mice. After oral administration to mice, most of the labelled Aridanin was found in the intestinal contents indicating a very poor absorption. In the snails incubated in labelled Aridanin, the radioactivity was extensively concentrated in comparison to the surrounding medium, and a retention was registered in many organs (kidneys, hepatopancreas, gastro-intestinal tract) up to 72-96h after incubation. The study however showed that the rapid uptake and disappearance and low oral uptake of Aridanin in a non-target animal (mice) and the accumulation and retention of Aridanin in target snails made this molluscicide a suitable substance for field studies (Adewunmi et al., 1989).
The effects of low concentrations of aridanin (0.125, 0.25, 0.5, and 1.0ppm) on the growth and egg production of *B. glabrata* and *Lymnaea columella* were compared with the effects of low concentrations of niclosamide (0.025, 0.05, and 0.10ppm). Aridanin and niclosamide was found by Adewunmi et. al. (1989) to cause significant reduction in the egg production and growth of the organisms, indicating that aridanin has a potential use in schistosomiasis control if used in slow release formulations. They also suggested that the poor stability of aridanin in clear water meant that its effectiveness as a molluscicide in the field will depend on the speed of its destructive action on snails.

A schistosomiasis control field project was carried out in 27 towns and villages in the South West Nigeria by Adewunmi et. al. (1990), which yielded data by which it was possible to relate snail recovery from potential transmission sites to the presence or absence of *T. tetraptera.* It was observed that a significant negative correlation with small numbers existed for distance of plants from transmission sites and fruiting of the trees when these variables were tested separately. There were no significant differences between individual variables such as P^{H}, calcium concentrations and temperatures for snail habitats but these variables produced significant positive correlation with the number of snails recovered. Thus the presence of this plant appeared to be the most important limiting factor for the presence of snails. The results thus confirmed *T. tetraptera* potential for the local control of schistosomiasis.

Adewunmi et. al. (1990) further demonstrated the mechanism of action of the major compound of *T. tetraptera*, aridanin and of serotonin on the rhythmicity of the intestinal smooth muscle preparations of *B. glabrata*. Methy-sergide and cyproheptadine antagonized the contractile action of serotomin. Venrapamil, lanthanum, and cyproheptadine inhibited the actions of aridanin, suggesting a calcium-dependent action of aridanin on gut tissue and that a prolonged administration of aridanin significantly decreased the uptake of available calcium by the snails.

Another insight into the mechanism of action of aridan and aridanin was provided by Adewunmi et al. (1991) which demonstrated that the molluscicides aridan, a water extract of *T. tetraptera,* and aridanin, a glycoside isolated from the fruit were tested for genotoxicity. It was substantiated that none of the substances had any influence on cell proliferation, and neither induced chromosomal aberrations, nor sister chromatid exchanges in Chinese hamster ovary cells cultured *in vitro,* suggested that aridan can be used as an agent in schistomiasis control without concern for genotoxicity.

Adewunmi (1991) also reported the effects of long-term treatment of eggs of *Biomphalaria glabrata* with aridanin and compared this with niclosamide by the treatment of 3-day old *B. glabrata* eggs with aridanin which caused a knock-down effect on the prehatch snails, making this group the most susceptible. The development and hatching of 0-to 1-day-old eggs could not be prevented but was prolonged by continuous exposure to aridanin (0.5-1-mg/L). Pre hatch snails were less susceptible than juvenile and adult snails, while niclosomide (0.625-0.35mg/L)

arrested the development of *B.glabrata*. The results therefore predicted a poor action of aridanin as an ovicidal agent in the control of snail intermediate hosts of schistosomiasis.

Experiments were conducted by Gebremedhin et al. (1994) with aridanin from *T. tetraptera* aridan and endod, an extract from *Phytolocca dodecandra* and niclosamide on non-target aquatic organisms such as leech, hydra, tadpoles, anopheline mosquito larvae and brine shrimps and compared with their toxicity to the target snail, *B. glabrata* at 0.04, 1.00, 30.00, and 40.00 ppm, respectively. It was observed that all the molluscicides killed the leech, a pest of animals and man at molluscicidal concentrations. The hydra and tadpoles were sensitive to the molluscicides except aridanin but the shrimps and anopheline mosquito larvae were resistant to all the molluscicides.

Noamesi et al. (1994) worked on the aqueous extract of the stem bark of *Tetrapleura tetraptera, Guibourtia ehie* and the root extract of *Taverniera abyssinica* used for the treatment of gastrointestinal related clinical proplems in Africa ethnomedicine. An Hcl/EtOH- induced ulceration was used in fasted rat stomach to investigate the anti-ulcertative properties of the extracts and an acute cytoxoxicity of the extracts using brine shrimp larvae was also investigated. The aqueous extracts of *T. abyssinica* (250-500 mg/kg), *T.tetraptera* (500-1000mg/kg), *G.ehie* (500-1000mg/kg) and a combination of *T. tetraptera* and *G. ehie* (1:4, 500-1000mg/kg) produced significant ($P<0.05$) inhibition (54-80%, 86-98%, 48-80% and 54-92% respectively) compared to controls. The acute cytotoxic concentrations of the extacts which killed 50% (LC_{50}) of brine shrimps within 24h was 438 mmg/ml for *T. tetraptera*, 220mg/ml for the 1:4 combination of *T.tetraptera* and *G. ehie* and 1409mg/ml for *T. abyssinica* and none of the shrimp were killed by *G. ehie*, even at 2mg/ml.

Experiments were conducted to determine the appropriate size of Aridan, *T. tetraptera* that could be used for efficient extraction of the molluscicide and the results demonstrated that both the particule size and concentration affected the molluscicidal activity of Aridan. In order to select a suitable particule size to massive Laboratory extraction of Aridan, a particle size of 186.5mm would be needed to achieve an ideal optimum and/or potent molluscicidal activity and that the adverse effects were few and transitory, necrosy also showed slight haemorrhagic appearance at the lungs. It was clearer that field testing of Aridan in schistosomiasis control was more justifiable. Awe et al. (1995).

In order to further confirm the mechanism of action opf aridan, Bode et al. (1996) also conducted a subchronic experiments with concentrations of saponins from *T. tetraptera* and Bayluscide to study the ultra structural effects of these molluscicides on *B. glabrata.* The ratio of the digestive cells to the crypt cells was inverted in molluscicide treated snails which showed an increase in the number of secretory cells and a decrease in the number of digestive cells. This digestive gland with dose-dependent autolysis of the membranous structures and the results showed that the molluscicides produce non-specific effects on the membranous structures.

In a similar vein, a study investigated whether water extract of *T. tetraptera* has trypanocidal effect against *T. brucei* in laboratory rats. This study also examined its effects on the weight, haematological parameters as well as the blood chemical analytes of the water extract to the infected rats significantly reduced the parasite load, weight loss was significantly lower in the infected and treated rats than the untreated ones. The results revealed that there was no significant difference in the concentrations of the blood glucose, total protein, uric acid and unconjugated bilirubin in the uninfected but treated rats compared with the neat rats which were neither infected nor treated which indicated that *T. tetraptera* may contain active substance against trypanosome infection, Okochi et al. (1999).

Some pharmacopoeial standards; acid-insoluble, water-soluble ash, as well as water-soluble extractive values of this plant in the South West of Nigeria were determined which were highest in the savanna zone and values of the commercial standards found to vary as: Savanna transitional fresh (rain forest frontier) > rain forest. The alcohol-soluble extractive was highest (6.63 + 0.20%) at derived Savanna zone of Omuo-Oke. The rank order of the alcoholic extractive was also found to be Omuo-Oke (savanna, 6.63 + 0.20%)> Ilugun/Olokemeji (derived savanna 6.46 + 0.12%)> Abata Egba (rain forest, 4.45 + 0.06%). The ecotoxicity of the plant showed that it was less toxic to *Thermocyclops oblongatus*, earthworms and *Paramecium* spp. then *Biomphalaria glabrata* but more toxic to cyprids, Clarias spp. and Epiplatys spp. (Adewunmi et. al., 2001).

The molluscicidal effect of nicotinanilide was evaluated and compared with niclosamide against different stages of the fresh water snail *Lymnaea luteola* eggs, immature, young mature, and adults and the calculated values of lethal concentration (LC_{50} and LC_{90}) showed that both nicotinanilide and niclosamide were toxic against eggs, immature, and adults. The young mature stage of the snails was comparatively more tolerant to both molluscicides than the other stages. The toxicity of the intermediate compounds of nicotinanitide against young mature stage at the snails was ineffective while the mortality pattern of the snails exposed to LC_{90} concentration of these

molluscicides showed inclosamide to kill faster (within 8 to 9h) than nicotinanitide (26 to 28h). It could therefore be concluded that both molluscicides were toxic against all stages of the *L. luteola*,(Sukumaran et al., 2004).

A study examined the anti-inflammatory and hypoglycaemic affected of *Tetrapleura tetraptera* fruit aqueous extract in rats. Fresh egg albumin-induced pedal oedema and streptozotocin (STZ)-induced *Diabetes mellitus* were used as experimental test models of inflammation and diabetes. *T. tetraptera* (50-800mg/kg p.o.) produced dose – related, significant reductions ($P<0.05$-0.001) of the fresh egg albumin – induced acute inflammation of the rat hind paw oedema. The plant extract also produced dose – dependent, significant reductions ($P<0.05$-0.001) in the blood glucose concentrations of both fasted normal and fasted diabetic rats. The results indicated that *T. tetraptera* fruit aqueous extract possesses anti-inflammatory and hypo-glycaemic properties. These findings lend pharmacological credence to the suggested folkloric uses of the fruit of the plant in the management and/or control of arthritis and other inflammatory conditions and adult-onset type 2 *Diabetes mellitus* in Yoruba-speaking communities of South-West Nigeria (Ojewole et al. (2004).

Adewunmi .(2004/2005) in his recent scientific appraisal of *T. tetraptera* itemised the potential uses of this plant with some of the corresponding phytochemicals; as molluscicide, anti-ulcer, antimicrobial, anticonvulsant, emulsifying property, birth control, intritive activities. Amoako-Atta *et al*. (2004/2005) upgraded the value of the indigenous biological food resources that abound in Ghana. In 1999, the Centre for Biodiversity Utilization and Development (CBUD) was established. This Dutch-funded programme has emerged as a Centre of Excellence with the mission to co-ordinate the process of identification of potential products of Ghana's biodiversity and to subsequently support and facilitate their production, processing and marketing. It has pursued this process of domestication and product chain development with five lesser-known food resources – snails, indigenous leafy vegetables, *Tetrapleura tetraptera* known for its medicinal and food value called "Prekese", a commercial product of Natu-Bi Preserve Establishment, a fruit juice canning company has made soft drinks called "Prekese" fruit (*T. tetraptera*) which is proclaimed/served as medicinal drink especially for the cure of hypertension; grass-cutter *(Thryonomis swenderianus*, a popular bush meat) and *Telfairia occidentalis*. It is worthy of note that Institutional arrangements already involved forty partner institutions across Ghana, and over 1,500 agricultural producers have taken up the production of the commodities promoted by the Centre throughout Southern Ghana.

A review of plants used for poison fishing in tropical Africa was done by Neuwinger, H.D. (2004) in which 325 fish-poisoning plants, spread among 71 plant families with 183 genera were presented. The closely related groups of Caesalpiniaceae, Mimosaceae and Papilionaceae clearly dominate. It was also remarkable that a great proportion were Euphorbiaceae. The plants most used are *Tephrosia vogelii, Mundulea sericea, Euphorbia tirucalli, Gnidia kraussiana, Adenia lobat, Balanites aegyptiaca, Swartzia madagascariensis, Neoratanenia mitis, Tetrapleura tetraptera* and *Strychnos aculeate*. It was shown that many fishing poisons play an important part in the preparation of arrow poisons and in traditional medicine as fishing with the aid of plant poisons has a long tradition all over the world and is still used in many places in the world today.

The petroleum ether, dichloromethane, methanol and water extracts from 24 plants, belonging to 19 families which were reported in the literature as traditional remedies for sleeping sickness (human African trypanosomiasis) were screened for *in vitro* activity against *Trypanosonia brucei,* as well as for cytotoxicity for a human fibro-blaster cell-line (WI – 38) along with the natural compounds berberine and harmane. A promising trypanocidal activity with IC_{50} values below 10mg/ml was found in 32 extracts of 13 plant species and the most active extracts with IC_{50} values below 1µg/ml were derived from *Annona senegalensis, Bussea occidentalis and Physalis angulata*. The plant extracts were found to show a modest selectivity index, in contrast to commercially available trypanocides with a more distinct selective toxicity against trypanosomes (Freiburghaus et al., 1996).

Ngassapa et al. (1993) isolated from the stembark of *T. Tetraptera* a known triterpene glycoside, 3-**0**- β-D-glucopyranosyl - (1´´-6´) – 2´– acetamido – 2´– deoxy-β-D – glucomyranosyl olean – 12-ene-28-oic acid, and a new sulphated triterpene, echinocystic acid-3-**0**-sodium sulfate. The –olean-12-ene-28-oic acid was found to be 100% lethal to *B.glabrata* at 20ppm, while the echinocystic acid-3-0-sodium sulfate was not molluscicidal at the same concentration. On the other hand, in a forward mutation assay utilizing *Salmonella typhimurium* strain TM677, *T. tetraptera* stembark extracts were found to be mutagenic in the absence of a metabolic activating system (S-9) while a MeOH extract of the fruit exhibited a weak mutagenic activity only in the presence of S-9. It was also found that the stembark isolates, aridanin, 3-0-(2´-acetamido-2´-depoxy-β-D glucopyranosyl) echinocystic acid, the-olean-12-ene-28-oic acid and the echinocystic acid-3-0-sodium sulfate were not mutagenic either with or without metabolic activation.

A very recent work was done by Aderibigbe et al. (2006) in which Aridanin isolated from *T. tetraptera* fruit was investigated for anticonvulsant, analgesic and hypothermic activities in mice. The results suggested that aridanin could be acting as a central nervous system (CNS) depressant and that its anticonvulsant property may be mediated through the membrane stabilizing property while the analgesic and hypothermic actions were mediated through opioids, cholinergic and 5-HT receptors respectively.

In a dosely related work of Aderibigbe et al (2006), aridanin was evaluated for neuropharmacological activity on novelty-induced behaviours such as locomotory, exploratory, stereotyped and hexobarbitone-induced sleeping time in mice. The results suggested that aridanin has a strong sedative and central depressant action but lacks psychopharmacological activities.

Conclusion

The necessary research in the field of vegetable molluscicides should be encouraged. Therefore, the use of plant molluscicides might be one of the best means for the control of schistosomiasis and trematode infections in third world endemic countries. Aridan *Tetrapleura tetraptera* is likely to cause less ecological damage and its potency of 1.33-5.22 ppm for different snails appears a promising one for a vegetable molluscicide as the most promising Nigerian plant molluscicide to date with the molluscicidal activity of the methanolic extract of the leaf, leaf-stalk, stem-bark, root-bark and fruit varies between 1.50 and 3.16mg/L indicating that all the parts are active.

The nutritive quality of the dry fruit of *T. Tetraptera* used as spice as assessed showed the fruit shell, pulp and seed contained varying amounts of nutrients such as protein, lipids and minerals, which were comparable and some higher than popular spices such as pepper, onion, curry and ginger a reason why it is used in the preparation of pepper soup in southern parts of Nigeria. The fruits also found to contain cinnamic acids, caffeic acid and carbohydrates (Adewunmi, 1999).

Studies on natural molluscicides should be well funded as a matter of urgency along with other tropical, neglected diseases like malaria, leishmaniasis, schistosmiasis, lymphatic filariasis and ochocerciasis, African trypanosomiasis and chagas disease, leprosy, dengue and tuberculosis, in laboratory, clinical, applied field research and social sciences disciplines. In support of this noble position, the European parliament recently has called for increased research into neglected diseases; which affect millions of people in the developing world but receive little attention from the global scientific community.

In this connection, the world desperately needs more research into diseases such as schistosomiasis that affect the poor because drugs for schistosomiasis and leishmaniasis, for instance, which affect millions in Africa, Asia and Latin America, are old and ineffective. It is important to translate research findings, including the genome sequencing of parasites causing malaria, leishmaniasis, schistosomiasis and African trypanomiasis (sleeping sickness) into new drugs. The world should equally engage in capacity-building efforts by training health-care workers and researchers if developing countries are to improve their basic health-care systems.

It is very pertinent to prospect for potent and environment friendly molluscicidal drugs from *Tetrapleura tetraptera* but the majority of the works to date has largely been achieved on biology or pharmacological activity and very few on the chemistry and there has not been any credible drug developed from this plant.

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