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# Significance of characterization of secondary metabolites from extracts of higher plants in plant disease management

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**ABSTRACT**

**Several sources reported the advantages of synthetic fungicides in boosting food production. However, there are serious concerns and negative consequences associated with their use on ecological and human health in recent years. Besides, pathogens' resistances to some of the most effective fungicides have been reported. These amongst other drawbacks prompted the search for alternatives. Plants offer an expanse of exploitable chemical space in this regard which is unparalleled in nature and not beaten by combinatorial chemistry as yet. Extracts of higher plants have demonstrated a wide range of activity against plant pathogenic organisms. These plants extracts have been found to contain broad spectra of phytochemicals (secondary metabolites) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, phlobatannins, polyphenols and steroids. Secondary metabolites constitute plants' weaponry against pests and pathogens invasion. These groups of phytochemicals possess wide ranging chemical functional groups; by which they establish contact with and bind to sites on target pathogens to ineffectuate them. Complexes of these secondary metabolites occur in crude extracts. Need therefore arises in the present to identify, isolate and purify the active secondary metabolite(s) in the crude extracts and to determine the one(s) effecting the reported kills and fungitoxity of the extracts. With the exception of neem assertive reports on the modes of action of most bioactive secondary metabolites are unavailable. We have to understand the functional groups of the bioactive phytochemical isolates, their position on the carbon skeleton of the principles, their binding sites on target pathogenic species; and the metabolic process(es) which they affect and ineffectuate. This will aid in either synergizing or synthesizing them so as to combat the enormous and obvious challenges of fungicide resistance threatening agricultural production and food security. This work reviews available literature in these regards.**



## INTRODUCTION

The National Bureau of Statistics (NBS) of the Government of Nigeria listed the important field crops grown in Nigeria. These crops include yam, cocoyam, cassava, cowpea, groundnut, sorghum, millet, maize, rice, melon and cotton. According to Abate et al. (2011), FAOSTAT (FAO Statistical Databases) included soybean as an emerging important field crop also grown in Nigeria. Statistics show that Nigeria is the world's largest

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producer of yams accounting for 70-76% of the world production. Currently she produces 35.017 million MT of yam valued at US$ 654 billion, on 3.045 million ha of farmlands (FAO, 2004; Kleih et al., 2012). Nigeria is the largest producer of cocoyam in the world, with 5.49 million MT of corms produced annually which accounts for 37-40% of the world's total (FAO, 2006). Similarly, statistics show that out of the 29.5 million MT of groundnut produced worldwide per annum, Nigeria contributes 1.51-2.57 million MT produced on 2.09 million ha of arable farmlands. The country also produced 0.53 million MT of soybean from 0.582 million ha (Abate et al., 2011; Soytech, 2011; Awurum and Uwajimgba, 2013). She is rated as the world's largest producer of the grain legume, cowpea. Singh et al. (2002) reported that Nigeria produces 2.0 million MT of cowpea grains on 5 million ha every year. Many socio-economic factors, and especially pests and disease pressures however; threaten the sustainable production, storage and preservation of these staple foods as well as several leafy vegetables, fruits and spices produced in the country (Awurum et al., 2001; Abate et al., 2011). These threats are projected to increase in the face of climate change challenges (Sadiku and Sadiku, 2011).

Fungi represent the greatest number of pathogens responsible for plant diseases and deteriorations (Ajibade and Amusa, 2001). Pathogenic fungi decimate agricultural crops or products at all stages of growth in the field, transit or storage (Amusa et al., 2003; Enyiukwu et al., 2013). It has been reported that up to 8000–750,000 crop diseases are caused by fungi with between 50–200 species, races and/or biotypes attacking a single crop (Ragsdale, 1994; Madden et al., 2008). Infection by fungi interferes with normal physiological function(s) of the host plant such as photosynthesis, biosynthesis, nutrient and water uptake among others, leading to great reductions in crop yield and product quality. Some pathogenic fungi such as *Aspergillus flavus* and *Fusarium moniliforme* produce toxins (*mycotoxins*) which cause cancer or may be nerve or organ poisons. These toxins make affected produce dangerous for human and livestock consumption (Ragsdale, 1994). The incidence of myco-pathogenic diseases upon a susceptible host-plant is dependent on the presence of specific conducive conditions of moistures in the atmosphere, in the soil and/or on the plant surface; in addition to optimum temperature for infection and disease development (Ragsdale, 1994; Amadioha, 2012).

Field phyto-fungal diseases may be nematode-assisted. Nematodes of the order *Xiphinema*, *Trichodorus, Longidorus and Para-longidorus* have been implicated in this regard. These stylet-possessing parasites puncture and burrow into tender, succulent plant roots sucking out vital growth factors from the infested crop, creating portals of entry for pathogenic fungi and bacteria. This consequently reduces the plant's anchorage and vigour, and predisposes the affected crop to damaging infections. For example, the yam nematode *Scutellonema bradys* and *Meloidogyne* spp. have been noted to attack yam in West, East and Central Africa (Coyne et al., 2003; Coyne et al., 2006; Mudiope et al., 2007). Cowpea on the other hand is seriously galled by members of the genera of *Meloidogyne* while *Practylenchus* sp. attacks plantains and bananas in the rainforest zone of the country (Amusa et al., 2003; Ononuju and Kpadobi, 2008).

Emechebe and Shoyinka (1985) reported that pathogenic fungi are capable of causing up to 100% yield loss in crops. Worldwide, it is projected that 20-40% of potential yield of crops are lost annually to plant diseases (Sygenta, 2012). This may aggravate to 50%, particularly in Africa due to climate change impacts, noted Sadiku and Sadiku (2011). It is estimated that globally these yield losses amount to between 60–525 billion US dollars annually (Agrois, 2004; Sygenta, 2012). In Nigeria alone, it is estimated that 1.5-20 million tonnes of cereals, tubers and legumes amounting to 0.3–0.4 billion Naira are lost in storage every year due to fungal attacks (Amadioha, 2012). In a bid to control these devastating attacks, synthetic fungicides are employed. Without controversy, fungicides contribute to yield increases in crop production. Adipala and Edema (1994) reported that the application of Mancozeb and Dithane M-45 significantly improved the yield of cowpeas in Uganda. In the USA, Bennett (2005) reported that 1.5 billion pounds of onion bulbs were harvested from onion plants cared for with less than 1 million pounds of fungicides. Economic use of fungicides in agriculture translates to improved returns on farm investment. According to this source, in California apple growers invested 70 million dollars on various fungicides, and reaped an attractive 1.2 billion dollars as return on investment (ROI). In general, a total of 880 million dollar-investment in fungicides by growers of different crops in the USA, all together reaped 12.8 billion dollars ROI; translating to US$1 investment in fungicides to US$14.6 financial returns in the farm economy. Fungicides play a very active role in production of high value crops with uniform appearances and quality (Biobank, 2009). Highly intensive and developed crop farming as practiced in the USA and Europe, involves use of highly-bred crop varieties to maintain uniform crop height, crop canopy, fruit size and shape as well as overall appearance and quality of produce in mechanized farms. Without fungicides and other pesticides it will be difficult to grow such crops of high horticultural characteristics in large monocultures given serious potential pathogenic challenges in the environment. In the US Agriculture, concluded Croplife (2012) many crops would not be produced commercially without fungicidal agents.

However, the high intensity of chemical pesticide applications and/or their inappropriate applications in

agriculture have become a serious cause of concern in recent years reported Biopesticides (2012). Several demerits obviously associated with use of these synthetic fungicides in agriculture and pest control programs have been reported such as pathogen resistance, pathogen resurgence, effects on non-target species, and ecological and human health concerns among others. Resistance to chemical agents is a very serious matter. Recently, FAO according to Par and Rajul (1994) noted 150 fungal pathogens to exhibit resistance to fungicides. Some authorities asserted that evolution of races and biotypes of pathogens to previously effective chemical agents occured 5-10 years post-introduction of the agent (Oreskes and Conway, 2010; Pallant, 2010). Besides, synthetic pesticides leave undesirable residues in the treated food materials and the environment. Some of these residues retain their toxic properties for a long time in the food chain; impairing metabolic processes when consumed by non-target species. These and many other factors gave impetus for alternatives to be sought (Awurum et al., 2005; Okwu et al., 2007; Amadioha, 2012). Some of the preferred alternatives are:

- Use of natural enemies of the pathogen such as *Trichoderma* sp., *Gliocladium* sp., *Bacillus thurengiensis* and *Baculoviruses* in managing challenges from the pathogen (Classwebs, 2012).
- Use of Intercropping to forestall and manage pests and pathogens problems in smallholder farms (Awurum et al., 2001).
- Farming systems re-design, through adopting crop rotation practices, proper field sanitation, good crop density, improved field aeration and furrow instead of overhead sprinkler irrigation systems etc. These obviously will minimize predisposing crops to fungal attacks (Abawi and Hunter, 1979; Anonymous, 2012).
- Use of botanical pesticides such as neem (Azatin, Bioneem, Tomco and Mangosan) and extracts of other higher plants to combat challenges from phyto-pathogenic organisms (Amadioha and Obi, 1998; Amadioha, 2002; 2003; 2004; Awurum et al., 2005).

This review focuses on use of botanical pesticides in plant health management. Many higher plants of the rain forest are being screened for fungicidal properties (Amadioha, 2001; 2002; 2003). Several workers have conclusively asserted the fungitoxic properties of these plant-based extracts for management of phyto-fungal diseases (Olufolaji, 1999; Awurum et al., 2005; Opara and Obana, 2010). These extracts have been reported to have the merits of being readily available in faming localities of the tropics, cheap, eco-compatible, less harmful to non-target organisms and useable in Integrated Disease Management (IDM) programmes for smallholder, resource-poor farmers. They are also reported to provide sustainable disease management solutions especially in organic farming where synthetic pesticides are non-tolerable. Plant-based extracts as pesticides requires no pre-harvest interval (PHI) limitations and leaves zero toxic load on treated produce compared to synthetic chemicals (STK, 2012). Above all, they are systemic (Opara and Obana, 2010) and contain multiple bioactive metabolites (Gurjar et al., 2012; Enyiukwu and Awurum, 2013). It is reasoned that to detoxify compound complex is more difficult than single molecules and this makes pathogens' resistance to plant extracts less likely (Adjaye-Gbewonyo et al., 2010; STK, 2012; Silva-Aguayo, 2013).

## USE OF PLANT EXTRACTS IN PLANT DISEASE CONTROL

Plants are the most important sources of chemical compounds known. Since 1763 ground tobacco (*Nicotinia tabacum*) has been utilized for control of pests of agricultural crops (Enyiukwu, 2011). Rotenone (*Derris* sp. and *Londocarpus* sp.) was discovered and used in pest control since 1850. These products are commercially available. Pyrethrins (*Chrysanthemium cinerariarium*) and their related synthetics have been commercially developed and used for pest control. Ever since several plant-based products such as Ryania (*Ryania speciosa*, Family: Flacontiaceae), sabadilla (*Schoenocaulon officinale,* Family*:* Liliaceae*)* with its synthetic derivatives as well as several neem-based products (Neemzal, Neem Gold, Ecocure, Tomco, Azatin) are commercially registered products available for pest management in some countries such as USA, Germany, India and the Americas. Neem contains 18 active principles principal amongst them being *azadirachtin* (Silva-Aguayo, 2013). At present many other botanical pesticides extracted from grape fruit seeds and garlic is also being marketed commercially as pest management chemicals. Extracts of the Chilean plant (*Calceolaria andina*, Family: Scrophulaviaceae) has attracted scientific attention as possessing strong pesticidal properties (Silva-Aguayo, 2013). Several compounds of plant origin including beberine, allicin, carvacrol, osthol, santonin and carvone have been registered as fungicides in China (Alanwood, 2013). Eco-PM (1.26% thyme and thyme oil, 0.06% clove) is a broad spectrum contact fungicide. It is reported to be very effective against powdery mildews, botrytis gray moulds, and Phytophtora late blight of garden vegetables and vines. It also controls black spot on roses, anthracnose and rust of ornamentals as well (Arbojet, 2013). Sabadilla (Lilliaceae) is reported to be a fungicidal product marketed in very many countries under several trade names. The product has low mammalian toxicity. It is effective against species of *Erwinia*, *Corynebacterium*, *Fusarium* and *Glomerella* and especially the safflower downy mildew pathogen

*Plasmopara halstedii*. The compound and its derivative disrupt cell membrane integrity and alter its function (Oros and Ujvalvy, 1999). According to STK (2012) Timorex Gold is a commecialy available natural fungicide extract for control of black sigatoka disease of plantains. The product is derived from *Melalueca alternifolia* and is marketed in many plantain growing countries of the world. It attacks the causal fungus of this disease at its 4 developmental stages better than synthetic fungicides which are reported to affect only 2 early developmental stages of the pathogen. Timorex Gold disrupts cellular integrity, increase membrane permeability in cell structures, leads to loss of cytoplasm and impairs respiration and ion transport process in affected organisms. Expectations therefore, are that within 10-15 years from now, the use of these compounds will increase attracting up to 25% of the world market share of pesticides (Silva-Aguayo, 2013).

According to Cowan (1999), initial screening of plants for antimicrobial activities; begin with their crude aqueous or alcoholic extracts. Early literature available to us indicated that extracts of wild *Ipomea carnea* and isolates of *Penicillin* sp. had fungitoxic effects against *Heminsthoporium oryzae*, *Heminsthoporium sativum* and *Colletotrichum capsici* which affect paddy rice, wheat and chilli respectively (Narian, 1970). Many plant extracts as pesticides have been reported to inhibit spore germination and mycelial growth of pathogenic fungi (Amadioha and Obi, 1998; Olufolaji, 1999; Mitali et al., 2012). These crude extracts were evaluated *in vitro* for activities against germination of the spores and mycelial growth of the pathogens, and later, for *in vivo* inhibition of the development and spread of the organisms in actual field conditions. Gurjar et al. (2012) argued that majority of these works on extract evaluations against pathogenic organisms were however conducted *in vitro*. The use of plant extracts in plant protection is summarized in Table 1. They have been found effective against seed-inhabiting pathogens, soil dwelling biotic agents, and nutrient and water uptake impairing organisms such as the wilt-inducing *Fusarium oxysporium* of egg plants. Others are root knot nematodes (*Meloidogyne* spp.) of okra as well as rots and various phyto-fungal pathogens of Amaranthus, legumes, tomato, yam and avocado etc. (Amadioha and Obi, 1998; Olufolaji, 1999; Amadioha, 2001; 2003). They have also proven effective for arresting the development and spread of bacteria-induced diseases of vegetables and tuber crops. Several plant extracts have been evaluated and found efficacious against pre- and post-emergence damping-off, post-harvest rots and transit-decay inciting pathogens of great implications in threatening post-harvest storage and preservation of produce in agriculture such as species of *Diplodia, Aspergillus, Botrytis, Botrydiplodia, Pythium, Fusarium, Mucor, Rhizopus, Penicillin, Sclerotinia, Alternaria, Rhizoctonia* and *Phytophtora* (Okigbo and Ogbonnaya, 2006; Sarpeleh et al., 2009; Gupta et al., 2012; Islam and Faraq, 2012). For example, recently phytochemicals from some tropical plants (*Carica papaya* and *Piper guineense*) strongly retarded the germination of spores of *Colletotrichum destructivum* (Enyiukwu and Awurum, 2011). Anukworji et al. (2012) reported the isolation of *Botrydiplodia theobromae* as the most virulent pathogen amongst other rot fungi from cocoyam corms in Southeast Nigeria and showed that extracts from *Allium sativum* and *Azadirachta indica* were fungitoxic against them. Similarly, Amienyo and Ataga (2007) indicated also that 30% strength of extracts of *Alchornea cordifolia* leaves reduced development of rot in mechanically injured and artificially inoculated sweet potato by the same organism to the tune of 46%. In an evaluation in sorghum, *Cymbopogon citratus* (30% strength) completely inhibited the growth of *Colletotrichum graminicola* and *Phoma sorghomi* causing seed and seedling rots in the plant (Somda and Sereme, 2007). Gupta et al. (2012) reported that extracts from *Eucalyptus terticornis* and *A. indica* improved seed germination and seedling vigour by decreasing the pre- and post emergence mortality and number of seedlings showing symptoms of black mould in attacked onion. Greenhouse studies conducted in Southeast Nigeria revealed that *P. guineense* and *C. papaya* extracts inhibited the development and spread of anthracnose caused by *C. destructivum* and the results compared well with a placebo (Enyiukwu and Awurum, 2013). In field trials, Awurum and Nwaneri (2011) and Awurum and Ogbonna (2013) reported extracts from *Dennettia tripetala* and *Spondias mombin* comparable in fungitoxic effects to benomyl in combating *Choanephora cucurbitarium* induced wet rot of *Amaranthus* vegetable. On-farm research depicted that while in Cameroun, Ambang et al. (2011) found methanoic extracts of *Thevetia peruviana* effective in controlling cercospora leaf spot (CLS) in groundnut, Awurum and Uwajimgba (2011) also found *Dennettia tripetala* fungicidal against *Fusarium* wilt of the same crop. Both reports compared well with benomyl treated plants. In Kenya, experiments on-farm on Common bean (*Phaseolus vulgaris L*.), indicated that neem inhibited *Fusarium* spp. in amended soils better than tobacco, Mexican marigold and periwinkle (Obongoya et al., 2010). There is something to note however, that different types of interactions occurred in soil amendment trials when extracts were mixed. The combinations of extracts involving 15 extracts from different families/genera were evaluated in Cameroun. The results showed antagonistic, synergistic and neutral effects. Clove and black pepper (1% concentration) combination for example, was found synergistic against mycelial growth and sclerotial formation of the pathogen *Sclerotium cepivorium*. Antagonism was observed in most combinations involving allspice and in the mixtures of clove and cinnamon at 0.5-3% concentration against the organism. However, in most clove mixtures with the

**Table 1.** Some important plant diseases controlled with extracts of higher plants.

| Disease | Crop | Pathogen | Extract used | Source |
|---|---|---|---|---|
| Bacterial blight | Egg plant | *Xanthomonas campestris pv vesicatoris* | *P. guineense,*<br>*Zingiber* sp.<br>*Allium* sp. | Opara and Obana (2010)<br>Opara and Wokocha (2008) |
| Anthracnose | Cowpea | *C. destructivum* | *P. guineense* seed,<br>*C. papaya* root, seed | Enyiukwu and Awurum (2011; 2012; 2013b) |
| Wet rot | *Amaranthus* | *Choanephora cucurbitarium* | *A. indica* bark.<br>*Dennetia tripetala* leaf,<br>*S. mombin* leaf | Olufolaji (1999)<br>Awurum and Nwaneri (2011) |
| Sclerotium stem rot | Cowpea | *S. rolfsii* | *A. meleguata* seed<br>*M. myristica* seed | Okwu and Njoku (2009) |
| Stem rot | Cowpea | *Rhizoctonia solani* | *P.guineense* leaf<br>*Cymbopogon citratus* leaf,<br>*Ocimum sp.* leaf.<br>*Ocimum sanctum.* | Amadioha (2001; 2012) |
| Blast | Rice | *Pyricularia oryzae* | *A. indica* seed oil. | Amadioha (2000; 2012) |
| Brown spot | Rice | *Cochliobolus miyabeanus* | *A. indica (*ethanolic extract*).* | Amadioha (2002) |
| Black pod | Cocoa | *P. palmivora* | *C. papaya* seed<br>*G. kola* seed | Wokocha and Nwogu (2008) |
| Mould | Mung bean | *A. niger* | *Vernonia* sp. leaf | Onuegbu (1996) |
| (Black) | Onion | *A. niger* | *Eucalyptus terticornia*<br>*A. indica* | Gupta et al. (2012) |
| Basal stem rot | Tomato,<br>Cowpea | *S. rolfsii* | *G. kola*<br>*Hyptis* spp.<br>*Citrus* peel | Wokocha and Okereke (2005)<br>Okwu et al. (2007) |
| | | *Curvularia lunata* | *D. stramonium* root,<br>*Colotropis procera* stem<br>*O. sanctum* leaf | |
| Stem rot | Vanilla | *F. oxysporium f. sp. vanilla* | *Eugenia aromatica* leaf<br>*P. bettle* leaf<br>*Alpinia galangal* rhizome<br>*Sphaeranthus indica* leaf | Suprapta and Khalim (2009.) |

exception of those with cinnamon and low doses of all spice single fungicidal effect (neutral) was observed (Montes-Belmont and Prados-Ligero, 2006). These evaluations in general, clearly demonstrated that the plant extracts from a wide range of families are potently efficacious in cultures, glasshouse studies and in field trials in inhibiting mycelial growth of diverse organisms, arresting their spores germination and development as

**Table 1.** Continued.

| Disease | Crop | Pathogen | Extract used | Source |
|---|---|---|---|---|
| Rot | Cashew-nut | *Aspergillus* spp.<br>*Trichodrema* spp.<br>*Cephalosporium* sp. | *Anacardium occidentalis* leaf<br>*Vernonia amygdalina* leaf | Suleman and Ogundana (2010) |
| | Musk bean, | *Phytophtora drechsleri*<br>*Verticillium diahlae* | *Perganum hamala* | Sarpeleh et al. (2009) |
| | Cucumber | *Scleretina scleroiorium*<br>*Aternaria* sp.<br>*Botrytis cinerea*<br>*Macrophomina phaseolus* | *(*shoot, flower, seed*)* | |
| Root rot | Cowpea | *Pythium alphanidermatum* | *Aloe vera* leaf*,*<br>*G. kola* seed<br>*A. indica,*<br>*Zingiber officinale* | Suleman and Emua (2009) |
| | Cowpea | *Pythium aphanidermatum* | *G. kola*<br>*Ziginber officinale* | Suleiman and Emua (2009) |
| | Maize/tomato | *Alternaria solani*<br>*F. moniliforme* | *Hermidesmus indicus*<br>*Withania sominifera*<br>*Rauwolfia tetraphylla.* | Sangvikar and Wadje (2012) |
| Post-harvest rot | Yam | | *Ocimum gratissimum* leaf<br>*A. meleguata* | Okigbo and Ogbonna (2006) |
| | Cassava | *A. niger*<br>*A. flavus*<br>*F. oxysporium*<br>*B. theobromae*<br>*Fusarium solani*<br>*Penicillin oxalicum* | *X. aethiopica*<br>*Zingiber officinale*<br>*A. meleguata* seeds<br>*A. indica* | Okigbo and Nmeka (2005)<br>Okigbo et al. (2009) |
| | Cocoyam | *Geotrichum candida*<br>*Cortichum rolfsii*<br>*S. rolfsii*<br>*A. niger*<br>*B. theobromae*<br>*F, oxysporium* | *O. basilium*<br>*V. amygdalina*<br>*A. indica* | Ugwuoke et al. (2008) |
| *Cercospora* leafspot | Cowpea | *Cercospora* spp. | *Dennettia tripetala* | Nwachukwu (2010) |
| *Curvularia* leaf spot | Maize | *C. lunata* | *Phyllanthus amarus*<br>*Tithonia diversifolia*<br>*Morinda lucida*<br>*Gliricidia sepium* | Akinbode (2010) |
| Damping off | Cowpea | *Scleretium rolfsii* | *Annona municata* | Chukwu (2010) |
| | Eggplant, Chilli pepper, Tomato | *Rhizoctonia solani*<br>*F. oxysporium*<br>*S. rolfsii* | *A. indica*<br>*A. sativum*<br>*Zingiber officinale*<br>*Allamonde* leaf | Islam and Faraq (2012) |

**Table 1.** Continued.

| Disease | Crop | Pathogen | Extract used | Source |
|---|---|---|---|---|
| Rice blast | Rice | *Pyricularia oryzae* | *Chloranthus japonica* roots<br>*Paulonia coreana* stem | Choi et al. (2004) |
| Rootknot nematode | Okra | *Meloidogyne* spp. | *A. indica* | Asawalam and Adesanya (2001) |
| Dry rot | Yam | *F. oxysporium,*<br>*A. niger.* | *Aloe babadensis* leaf,<br>*Nicotinia tabacum* leaf,<br>*A. indica* leaf. | Taiga (2009, 2011) |
| Seed rot (dry) | Melon | *Rhizopus stolonifer*<br>*Penicillium italicum*<br>*A. niger* | *O. gratissimum*<br>*A. indica* | Chuku et al. (2010) |
| | Sorghum | *Colletotrichum gramminis*<br>*P. sorghumi*<br>*F. moniliforme* | *C. citratus*<br>*Eucalyptus camaldulensis* | Somda and Sereme (2009) |
| | Potato/ Vegetables | *Rhizoctonia bataticola*<br>*Rhizoctonia solani*<br>*A. flavus*<br>*A. niger* | *Aloe vera*<br>*Azadiractha indica*<br>*O. sanctum* | Gurjar and Talwankar (2012) |
| Brown spot | Rice | *Bipolaris oryzae* | *Nerium oleander* leaf<br>*Callistemon citrinus*<br>*O. gratissimum.* | Harish et al. (2008)<br>Nguefack et al. (2007) |
| Fruit rot | Pawpa | *A. niger*<br>*B. theobromae*<br>*F. solani*<br>*Penicillin* sp. | *C. papaya*<br>*Chromolaena odorantum*<br>*Acalypha ciliate* | Ilondu (2011) |
| White rot | Onion | *Sclerotium ceporium* | *Pimenta dioica*<br>*Syzygium aromaticum*<br>*Piper nigrum* | Montes-Belmont and Prados-Ligero (2006) |
| Wilt | Brijal | *Fusarium oysporium f.* sp. *melongae* | *A. indica*<br>*Artemisia annua*<br>*Eucalyptus glabulus*<br>*O. sanctum*<br>*Rheum emodi* | Babu et al. (2008) |
| | Tomato | *Fusarium oxysporium f.* sp. *lycopersici* | *A. indica* kernel, stem, leaf | Agbenin and Marley (2006) |
| Brown blight | Tea | *Glomerella cingulata* | *Pongamia pinnata*<br>*Syzigium aromatia*<br>*Alcorous calamus*<br>*Ageratum conyzoides*<br>*A. sativum*<br>*Abutilon indicus* | Kuberan et al. (2012) |

**Table 1.** Continued.

| Disease | Crop | Pathogen | Extract used | Source |
|---|---|---|---|---|
| Blight | Wheat | *Bipolaris sorokiniana* | *Adhatoda vasicola*<br>*Zingiber officinale* | Nagis et al. (2012) |
| Seed-borne diseases (rot, seedling blight, Bipolaris leaf spot, curvularia leaf spot) | Maize | *F. oxysporium*<br>*F. moniliforme*<br>*Penicillin* spp<br>*Aspergillus* spp.<br>*Bipolaris maydis*<br>*C. lunata*<br>*Rhizoctonia stolonifer* | *A. sativum*<br>*A. indica* | Debnath et al. (2012) |
| Seed/seedlng rot | Soybean | *Cephalosporium acromonium*<br>*Rhizopus leguminosa*<br>*Colletotrichum dermatium*<br>*Macrophomina phaseolina*<br>*Phoma* sp.<br>*S. rolfsii*<br>*C. lunata*<br>*F. oxysporium*<br>*Penicillin chrysogenum*<br>*Mucor mucedo*<br>*A. alternate*<br>*A. flavus*<br>*Aspergillus fumigatus*<br>*A. niger* | *Acacia nilotica*<br>*A. indica*<br>*D. stramonium*<br>*Polyathia longifolia*<br>*Annna squamosal*<br>*A. sativum* | Rathod and Pawar (2012) |

well as spread of pathogenic diseases from both artificially inoculated plants and naturally infected plants in actual field conditions.

Plants extracts as seen from Table 1 are hence suitable for exploitation as potent sources of pesticides to reduce losses arising from pathogenic attacks on crops and stored products (Amadioha, 2012). The use of these natural products for pathogenic disease management is particularly important and necessary in the developing economies of the world like Nigeria where synthetic fungicides are not only unavailable but farmers who produce about 98% of food in the country are poorly equipped to handle them making their use uneconomic for resource-poor farmers.

## Phytochemical evaluation of extracts

It has been noted that, despite the use of plant extracts in ethno-medicine, African cuisines and recently in plant protection, that their phytochemical composition and active ingredients have not been fully documented (Okwu and Njoku, 2009). Corroborative studies estimated that only 4 -10% of the 250,000 plant species constituting the biodiversity of the world's flora have been examined chemically for antimicrobial activity (Earnsworth, 1990; Pallant, 2010). A huge potential therefore exists in this regard especially in the tropical areas like Nigeria; with vast untapped rainforest, for these fungitoxic plant species to be extensively examined chemically. Scientific analysis of plants it has been observed follows a logical pathway beginning with a lead from the natives. Hence, Cowan (1999) reported further that initial aqueous and alcoholic screening of plants extracts for antimicrobial activities are followed by other organic extraction methods for determination of their phytochemical compositions. According to Wessells and Hopsons (1988), marvelous assortments of chemicals which are noxious to pathogens and even pests, have been found to be present in plants. These antimicrobial constituents (phytochemicals) include alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides, anthraquinones,

**Table 2.** Phytochemical composition of some plants materials used in plant protection.

| Plant Materials | Phytochemical constituents *(%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Alka** | **Flav** | **Tan** | **Sap** | **Phe** | **Ter** | **Gly** | **Ste** | **Source/refernce** |
| *Ricimus communis* leaf | - | + | + | + | + | - | + | + | Yadav and Agarwala (2011) |
| *Xanthium strumarium* leaf | + | + | + | + | + | - | + | - | Yadav and Agarwala (2011) |
| *Tinospora cordifolia* | + | + | + | + | + | + | + | + | Yadav and Agarwala (2011) |
| *Hyptis sauveolens* | + | - | + | + | - | + | + | - | Pachkore et al. (2011) |
| *Clutia abyssinica* | - | + | - | + | + | + | - | - | |
| *Euphobia hirta* | + | + | + | + | + | + | + | - | Ibrahim et al. (2012) |
| *Eruca sativum* | 11.27 | *24.43* | 4.15 | 6.20 | 26.90 | - | 2.76 | - | Mohammed et al. (2011) |
| *A. cordifolia* leaf | 5.90 | *4.20* | 6.80 | | 3.20 | | | | Adeshina et al. (2012) |
| *Cleome rutidosperma* | 0.34 | *0.34* | 15.25 | 2.00 | 0.20 | - | + | - | Edeoga et al. (2005) |
| *Emillia coccinea* | 0.92 | *0.96* | 11.85 | 2.30 | 0.81 | + | + | + | Edeoga et al. (2005) |
| *Euphobia heterophylla* | 0.86 | *0.74* | 12.46 | 0.00 | 0.10 | + | + | - | Edeoga et al. (2005) |
| *Physalis angulate* | 0.40 | *0.15* | 13.15 | 3.92 | 0.80 | + | + | + | Edeoga et al. (2005) |
| *Richadis brasilienss* | 0.45 | *0.56* | 12.13 | 1.12 | 0.14 | + | + | + | Edeoga et al. (2005) |
| *Scorpania dulchis* | 0.81 | *0.88* | 6.23 | 0.00 | 0.04 | + | + | - | Edeoga et al. (2005) |
| *Sida acuta* | 1.04 | *0.98* | 6.08 | 0.00 | 0.08 | - | + | - | Edeoga et al. (2005) |
| *Spigella anthemia* | 0.84 | *0.77* | 15.05 | 2.26 | 0.10 | - | + | + | Edeoga et al. (2005) |
| *Stachytaphyta cayennensis* | 0.68 | *0.00* | 9.98 | 3.10 | 0.13 | - | + | - | Edeoga et al. (2005) |
| *Tridax procumbens* | 0.58 | *0.61* | 7.45 | 1.70 | 0.06 | - | - | - | Edeoga et al. (2005) |
| *C. papaya* seed | 0.62 | *0.34* | 0.22 | 0.68 | 0.08 | - | + | - | Enyiukwu and Awurm (2013) |
| *C. papaya* root | 0.75 | *0.57* | 0.34 | 1.40 | 0.05 | + | + | + | Enyiukwu and Awurm (2013) |
| *P. guineense* seed | 1.63 | *1.23* | 0.88 | 2.64 | 0.66 | + | - | + | Enyiukwu and Awurm (2013) |
| *M. myristica* leaf | 4.28 | *8.29* | 0.34 | 0.02 | 0.03 | | | | Okwu and Njoku (2009) |
| *M. myristica* seed | 0.41 | *0.12* | 0.03 | 0.87 | 0.02 | | | | Okwu and Njoku (2009) |
| *Bryophyllum pinnatum* | 1.48 | *1.72* | 0.51 | 1.74 | 0.06 | | | | Okwu and Uchenna (2009) |
| *Cissis populnea* root | 2.79 | *4.13* | 1.18 | 1.11 | - | 0.36 | 0.53 | 0.13 | Soladoye and Chukwuma (2012) |
| *C. populnea* stem | 4.70 | *1.46* | 1.10 | 1.21 | - | 0.27 | 0.41 | 0.15 | Okwu and Uchenna (2009) |
| *Cassia alata* seed | 3.24 | *0.50* | 2.46 | 6.44 | 0.95 | | | | Okwu and Uchenna (2009) |
| *Nauclea latifolia* leaf | 4.32 | *0.36* | 0.01 | 0.98 | 0.06 | | | | Okwu and Uchenna (2009) |
| *N. latifolia* seed | 0.59 | *0.56* | 0.06 | 1.34 | 0.05 | | | | Okwu and Uchenna (2009) |
| *N. latifolia* fruit | 0.28 | *0.81* | 0.01 | 0.42 | 0.02 | | | | Okwu and Uchenna (2009) |
| *A. indica* leaf | 0.52 | *0.62* | 9.10 | 2.10 | 0.02 | | | | Khrishnaiah et al. (2009) |
| *Molinger oleifera* | 0.36 | *0.51* | 9.20 | 2.30 | 0.08 | | | | Khrishnaiah et al. (2009) |
| *Cleredendon* sp. leaf | 5.41 | *0.70* | 3.60 | 2.10 | 0.08 | | | | Okwu and Uchenna (2009) |
| *S. mombin* leaf | 6.00 | *3.00* | 3.80 | 7.60 | 1.00 | | | | Njoku and Amaefula (2007) |
| *Afromonum meleguata* leaf | 0.29 | 2.15 | 0.16 | 0.14 | 0.10 | | | | Okwu and Njoku (2009) |
| *A. meleguata* seed | 5.64 | *5.78* | 0.39 | 1.24 | 0.11 | | | | Okwu and Njoku (2009) |
| *C. odorantum* | - | - | 1.98 | 0.38 | - | 0.13 | | | Igboh et al. (2009) |
| *Citrus limonum* | 0.54 | *0.64* | 1.31 | 0.34 | 0.25 | | | | Okwu et al. (2007) |
| *Detarium senegalese* | 0.72 | *5.68* | 0.79 | 4.60 | 0.25 | | | | Uchegbu and Okwu (2012) |
| *Uvaria chamae* | 0.81 | *5.70* | 0.40 | 0.38 | 0.10 | | | | Okwu and Iroabuchi (2009) |

*, qualitative representation; +, indicate presence of constituent; -, indicate absence of constituent.

coumarins, polyphenols, Phlobatannin and steroids (Wessells and Hopsons, 1988; Edeoga et al., 2005; Okwu and Njoku, 2009; Soladoye and Chukwuma, 2012). The phytochemical constituents of some plant materials used in plant protection are presented in Table 2. They may be qualitatively represented or quantitatively documented (Edeoga et al., 2005; Jeruto et al., 2011). In a qualitative evaluation, Adebayo et al. (2009) reported the absence of alkaloids and anthraquinones from the leaves, stem bark and root of *Ficus exasperate*.

Quantitative documentation of phytochemicals in plant materials are presented in mg/100g of dry weight of specimen, g/100g of weight of the specimen (Aliyu et al., 2008; Harisaranraj et al., 2009; Okwu and Iroabuchi, 2009; Senthilkumar et al., 2011) or as percentages of weight per volume (w/v) of extract (Edeoga et al., 2005; Okwu et al., 2007; Uchegbu and Okwu, 2012; Enyiukwu and Awurum, 2013). This review will only present results expressed as percentages. Studies show that sometimes there may be disparity in the reported yield values of these phytochemicals by different investigators. For instance, Enyiukwu and Awurum (2013) reported 1.63% alkaloids present in the seeds of *P. guineense* which figure differed hugely from the value 5-8% noted in Purseglove (1976). This difference in values the authors attributed to influences from time of harvesting of the plant materials, extracting solvent, and method of extraction. The latter two factors seem most important to the reviewers. Variation in extracting methods are usually dependent on the length of the extraction period, solvent used, solvent pH, temperature, particle size of plant material and the solvent-to-sample ratio. According to Gurjar et al. (2012), the finer the particles size of the specimen the higher and better the rate of extraction. Furthermore, in a study Eloff (1998) found that 5 min extraction of very fine particles of diameter 10 µm gave higher quantities of phytochemicals than values obtained after 24 h in a shaking machine with less finely ground materials. Later investigations revealed that solvent-to-sample (solvent to dry weight) ratio of 10:1 had proved ideal for extraction of phytochemicals (Green, 2004; Gurjar et al., 2012). The observed differences in the yield of phytochemicals amongst parts (leaf, stem, bark, flower, seed) of the same plant, the authors further added may be occasioned by factors of age of the plant, plant part, sex and cultivar used in the investigation. Enyiukwu and Awurum (2013a) noted that in a study at Cornell University, that male *Carica* plants yielded more phytochemicals than female ones and older plant parts yielded more than the young ones. According to Silva-Aguoye (2013) high concentrations of phytochemicals are found in flowers and seeds of assayed plants. Similarly, it was also noted from the same Cornell University study that the potency of activity of the yielded phytochemicals were age of plant or plant part dependent. For instance, phytochemicals from young plants or plant parts were observed to be more active than those from older plants or plant parts. Variations in yield values of phyto-chemicals may also be occasioned by the climatic and edaphic variations in the geographic locations of growth of the plant (Pallant, 2010).

Phytochemicals of the group alkaloids have complex structure. They are bitter tasting, colourless, basic and toxic; and contain nitrogen in a heterocyclic ring. At room temperature alkaloids may be liquids or crystalline solids (Okigbo et al., 2009). They are the most physiologically active compounds of medical importance found in plants. Alkaloids and their derivatives are used as basic starting points for drugs. They possess antifungal and bactericidal properties (Okwu and Uchendu, 2009). Karlovsky (2008) reported that alkaloids can inactivate enzymes, block ion channels, interfere with neurotransmission and cause loss of electrical coordination (ataxia) in affected organisms.

Flavonoids are polyphenolic compounds possessing 15 carbon atoms made up of two benzene rings joined by linear carbon chain. They represent the most common and widely distributed class of plant phenolics. Flavonoids are a class of secondary metabolites known most commonly for their antioxidant and free radicals scavenging activities. Aside of preventing oxidative cell damage; flavonoids also play roles in combating allergies and microbes (Okigbo et al., 2009). Some flavonoids of the sub-class isoflavonoids exhibit high pesticidal activity. This happens through anaesthetic-like action related to electron transport blockade in the mitochondria brought about by inhibiting oxidation linked to $NADH_2$ (Freidli, 2008; Okwu and Njoku, 2009).

Tannins comprised of a large assemblage of natural products which have unpleasant taste and are employed in tanning leather (Okigbo et al., 2009). They have great natural diversity and generally are made up hydrolysable and condensed tannins (Okwu and Njoku, 2009). They exhibit pain killing properties. Tannic acids are used for prevention of loss of plasma and limiting secondary infection in burn wounds. Tannins-rich plants extracts are used by Asian natives for the treatment of ulcers. This class of phytochemicals has uniquely high affinity for precipitating proteins and complexing with all kinds of biomolecules (Peru, 2001; Dharmananda, 2007). Phenols otherwise called carbolic acids are aromatic alcohols consisting of a benzene ring bonded directly to a hydroxyl group (OH) (De Ruiter, 2005). They are weakly acidic and have long history of roles in antisepsis and disinfection (Okigbo et al., 2009). They are used as the starting ingredients in the industrial production of drugs, herbicides, synthetic resins and additives to inhibit microbial growth in various ranges of pesticides (Greener Industry, 2009). Phenolics slow growth, block microbial cell division and enzyme activity. According to Okwu et al. (2007), they caused swelling of fungal hyphae tips, plasma seeping and leaking around hyphae tips; cell wall distortions, abnormal branching or fusion of hyphae surface.

Saponins are glycosides of both triterpenes and steroids known for the soap-like foaming they produce in aqueous solutions. Saponins have a characteristic property of being bitter or astringent (Okigbo et al., 2009). Their soap-like nature makes them useable as surfactants and adjuvants for vaccines to enhance macromolecule penetration (Enyiukwu and Awurum, 2013). Saponins can ward off microbes and this makes

them good candidates for treating yeast, viral and fungal infections. They are known to play a role in cytolysis by complexing with cell membrane bilayers (Okwu and Njoku, 2009) sometimes creating pores on them (Rongai et al., 2012).

In recent years, noted a study from Italy that plants of the family of *Brassicacaea* have attracted scientific attention due to their high contents of glucosinolates. These glycosidic compounds have no biocidal activity in their native forms but are converted through enzymatic hydrolysis within living systems to their active forms (isothiocyanates) which have strong cytotoxic activity (Rongai et al., 2012). Methyl isothiocyanate (MITC) and benzyl isothiocyanate (BITC) was reported present in *C. papaya* (Morton, 1987; Cornell University, 2012). BITC occurs in the range of 1.37–1.96% in the *Caricaceae* (Tang et al., 2001). According to Reulas et al. (2003), BITC isolated from Papaya seeds is known to be a strong antifungal compound. At 0.5 mg/ml the compound inhibited the fungus *Alternaria alternata* which causes post-harvest rot of tomato. Later *in vitro* evaluations indicated that aqueous seed extract from *C. papaya* was superior to metalaxyl (ridomil) in inhibition effects against *Phytophtora palmivora* which incites black pod disease of cocoa. Investigators believed BITC was responsible for this reported activity (Wokocha and Nwaogu, 2008; Enyiukwu and Awurum, 2013a). Furthemore, the efficacy of *Afromonium meleguata* and *Monodora myristica* seeds and leaves extract against *Sclerotium rolfsii*, the incitant of basal stem rot of cowpea; according to Okwu and Njoku (2009) were due to their high contents of alkaloids. Enyiukwu and Awurum (2011; 2012), also attributed the potency of *P. guineense* seed extracts in the inhibition of spore germination, mycelial elongation, development and spread of the causal organism of anthracnose (*C. destructivum*) of cowpea in culture and glasshouse, largely to its high contents of alkaloids and perhaps, terpenoids in the test seeds extracts. Similarly, alkaloids are also reported responsible for the fungitoxic activities of extracts of *Raecimus communis* and *Datura stramonium* against several phytopathogenic fungi, while *Cucumina longa* and *A. indica* contain bioactive terpenoids which have been implicated for activities against a broad array of plant pathogenic fungi and bacteria (Gurja et al., 2012). Amadioha and Obi (1998) in a study with spices suspected and attributed the potency of extracts of *Xylopia aethiopica* againt *C. lindemuthianium*, the incitant of anthracnose of cowpea to perhaps be the presence of xylopic acids in the plant materials.

## Functional chemical groups

In a study on cocoyam in Nigeria, it was inferred that rot causing fungi produce pectinolytic and cellulolytic enzymes which degrade cell wall polymers and as such make available carbon sources for the invading pathogens. *Ocimum basilium* extract which inhibited the rot-causing fungi in the study was seen as possessing active principle(s) that arrested the ravages of the pathogens or their enzymes or both (Ugwuoke et al., 2008). Tests with infra-red reveal that these phyto-chemicals, according to several workers, contain many chemically potent functional groups. Functional groups are specific groups of atoms or bonds within molecules that are responsible for the characteristic chemical reactions of the molecules. Same functional groups are known to undergo same or similar chemical reaction regardless of the size of the molecule it is a part of. However, its relative reactivity is modified by nearby functional groups. Functional groups include amides, carbonyls, esters, aldehydes, phenyls, hydroxyls, ethyls, methyls etc. With these functional groups extracts (isolates) establish bonds with target enzymes, hor-mones; organelles or processes of pathogens to their harm (Brown, 2006; Okwu and Ukanwa, 2010b). Echeverrigaray et al. (2010) reported that hydroxyl and carbonyl groups or substituents on carbon skeletons of various monoterpenes are responsible for their inhibitory activity against soil inhabiting nematodes. The report further suggested that in addition, the position of these functional groups also influence the observed activity. Malheiros et al. (2005) in agreement from a parallel study, reported that dramane sesquiterpenes from *Drimys brasilliensis* inhibited a variety of human myco-pathogens including the stubborn *Epidermophyton floccosum*; and showed that their activity decreased 8 times (from 3 mg/ml to 25 mg/ml) when a bulky substituent (*p*-methoxy or *p*-hydroxycinnamoyl) is present in carbon position 1. Some of the essential functional groups of phytochemicals are listed in Table 3.

According to Okwu and Uchenna (2009), many other functional groups exist in these phytochemicals besides the ones listed in Table 3, such as pyrones, double and triple bonds etc. These chemically reactive groups have been postulated to be isolates' arsenals of attack against metabolic sites and enzymes of pathogens (Okwu and Ukanwa, 2010b) and even host plants in the case of phytotoxic chemicals (Echeverrigaray et al., 2010).

## Characterized isolates of some extracts

Plant-based pesticides remain typically on the surfaces of crops about 24 h post-application. They are reported to be decomposed rapidly by UV light and high temperatures of the hot tropics. This is the single most important drawback of their use in agriculture. This singular demerit gave the most impetus for the industrial modification of efficacious isolates/compounds of plants origin to make them more persistent and to sustain their

**Table 3.** Infra-red analysis of functional groups of *A. meleguata* and *M. myristica* isolates.

| Plant Isolate | Frequency | Functional group | Compound type | Reference |
|---|---|---|---|---|
| *Afromoniun meleguata* seed | 3413.07 | OH | Hydroxyl phenol | |
| | 3108.08 | C-N | Amine | |
| | 2925.88 | C-H | Aliphatic stretching | |
| | 2854.04 | C-H | Aliphatic stretching | |
| | 1709.15 | C=O | Carbonyl ester | |
| | 1654.99 | C=O | Carbonyl ketone | |
| | 1604.88 | C=C | Aromatic | |
| | 1121.00 | C-O | Ether | |
| | 3418.90 | O-H | Hydroxyl phenol | |
| | 2924.00 | C-H | Aliphatic stretching | |
| | 2852.41 | C-H | Aliphatic stretching | |
| | 1738.83 | C=O | Carbonyl ester | |
| | 1615.89 | C=C | Aromatic | |
| | 1455.75 | C=C | Aromatic | |
| | 1376.98 | C=C | Aromatic substitution | |
| | 1164.45 | C-O | Aromatic substitution | |
| *A. meleguata* leaf | 3401 | O-H | Hydroxyl phenol | Okwu and Njoku (2009) |
| | 3008 | C-N | Amine | |
| | 2926 | C-H | Aliphatic stretching | |
| | 2864 | C-H | Aliphatic stretching | |
| | 1464 | C=C | Aromatic | |
| | 1378 | C-O | Ester | |
| | 1244 | C-O | Ether | |
| | 1177 | C-N | Amine | |
| | 1098 | C-O | Ether | |
| | 1050 | =C-H | Aromatic substitution | |
| | 801 | =C-H | Aromatic substitution | |
| | 723 | =C-H | Aromatic substitution | |
| *M. myristica* seed | 3442.25 | O-H | Hydroxyl phenol | |
| | 2359.42 | C-H | Aliphatic hydrocarbon | |
| | 1634.29 | C-=O | Carbonyl | |
| | 1539.26 | C=C | Aromatic | |
| | 1455.90 | C=C | Aromatic | |
| | 668.13 | =C-H | Aromatic substitution | |
| *M. myristica* leaf | 3421.22 | O-H | Hydroxyl | |
| | 2854.11 | C-H | Aliphatic stretching | |
| | 2360.52 | C-H | Aliphatic stretching | |
| | 1735.85 | C=O | Carbonyl ester | |
| | 1653.09 | C=O | Carbonyl ester | |
| | 1558.43 | C=O | Carbonyl ketone | |
| | 1507.08 | C=O | Carbonyl ketone | |
| | 1457.81 | C=O | Carbonyl ketone | |
| | 1375.28 | C-O | Ether flavonoid | |
| | 1260.39 | C-O | Ether flavonoid | |
| | 1022.31 | C-O | Ether flavonoid | |
| | 799.60 | =CH | Aromatic substitution | |

toxicity against susceptible target species of pathogens (Silva-Aguayo, 2013). For example, nicotine and pyrethrins have been successfully modified into longer acting, weather tolerant, semi-synthetic laboratory copies called pyrethroids and neo-nicotinoids respectively. These products are highly effective in hot tropical environments (Brown, 2006). Therefore proper elucidation of the active principles of an extract has been realized in these recent times and considered as important tool towards maximizing the efficacy of the bioactive ingredient.

The effectiveness of the activity of phyto-chemicals against pathogenic organisms has been reportedly suggested to depend on the type and concentration of bioactive principles they contain (Owolade and Oshikanlu, 1999). In India, Stripathi and Poongothai (2010) reported that from bioassay-guided fractionation of *Pisonia grandis* a tropical medicinal plant inhibited the fungus *Monascus purpureus* better than clotrimazole a standard drug against its infections in humans. Of all the plant materials listed in Table 1 reportedly used in many researches on toxicity to phyto-parasites, only neem (*A. indica*) has been most extensively studied and characterized. Neem has been asserted to contain 18 active principles including the terpenoids azadirachtin, azadiradione, epoxy-azadiradione, nimbin, solannin, 6-diacethyl-nimbin amongst others. Azadirachtin is the most potent of the components and possesses strong antioxidant properties (Da Costal et al., 2010; Silva-Aguoye, 2013). Scott et al. (2005) also documented that *P. guineense* contain the alkaloids piperine and piperidine which have strong fungitoxic and pesticidal attributes. Allicin isolated from *Allium* spp. effectively controlled seed-borne *Alternaria* spp. in carrot, *Phytophtora* leaf blight of tomato and tuber blight of potato. Guie et al. (2003) indicated the isolation of flavanol glycoside *isorhamnetin* from *A. cordifolia.* Later investigation revealed the presence of anthocyanidine glycoside also in *A. cordifolia*, and this compound was fingered to underpin its antibacterial, antiviral and antimicrobial properties (Okwu and Ukanwa, 2010b). Polygodial (sesquiterpenes) has been isolated from *D. brasilliensis* (Malheiros, 2005). The compound has been found to exhibit insect antifeedant, antimicrobial activities. It also showed fungicidal activities to yeast, filamentous fungi and the medically difficult to eradicate endomycotic *E. fluccosum* (Taniguchi et al., 1988; Lunde and Kubo, 2000; Malheiros et al., 2005). Recently a few other plant materials have been examined chemically and their active principles isolated, characterized and documented Table 4.

Plants contain a marvelous array of potent and bioactive chemical compounds which play roles in warding off microbial, pest and herbivores attacks. A source noted that 12, 000 of such chemical compounds have been isolated from the plant kingdom. In the thoughts of Pallat (2010), these are still infinitesimal compared to the enormous chemical space provided by the 250,000 species constituting the world's flora. Identification, isolation, characterization and purification of novel potent compounds of plant origin will pave way to sustainable food production. It will reduce post-harvest contaminations and losses arising from pathogenic attacks on agricultural produce in transit and store. In addition, it will help to delay or reverse pathogen's resistance to plant health management chemicals.

**Mode of action of isolates from biopesticides**

Mechanism of action (MOA) of a chemical substance refers to the specific biochemical interaction through which a chemical substance exerts or produces its effects. MOA includes a crystal clear mention of specific molecular targets to which the chemical binds such as enzymes or receptors. For example, in a related field of pharmacology, it is known that salicylic acid, acts by irreversible inhibition of the enzyme *cyclo-oxygenase* and suppressing the production of the hormones prosta-glandins and thromboxanes leading to pain relief. The demand for bio-pesticides especially botanicals however, are seriously constrained by dearth of knowledge of their modes of action, in particular their target specificity and slow action. These have put them in a serious disadvantage *vis-a-viz* the synthetic chemicals noted for their fast action. Despite the glorious potentials of plant extracts for crop disease management, they are also demerited in being photo and themo unstable. Like their predecessors the *pyrethrins, nicotine and rotenone* they have short half-life and shelf storageability being rapidly degraded by high temperatures and ultra-violent radiations (Kumar, 1986; Eno, 2011; Gurjar et al., 2012). Aside of the pyrethrins (sodium channel modulators), nicotine (acetylcholine agonist) and rotenone (electron transport inhibitor at *cytochrome A*) whose modes of action have long been determined; of all the plant materials above, only *azadirachtin* isolated from neem has been extensively studied. The compound is reported to possess strong antioxidant activity which feature enables it to inhibit aflatoxin production in mycotoxigenic fungi (Da Costa et al., 2010). Azadirachtin acts clearly by infringing on the activities of cytosolic enzymes of fungi and antagonizing prothoracicotropic hormone (PTTH) of target pest organisms (Brown, 2006; Da Costa et al., 2010). From available literature, many reports only offer postulations on the modes of action of these bioactive metabolites. For example, in a parallel study, Okwu and Ukanwa (2010) suggested that anthocyanidine glycoside from *A. cordifolia* inhibited *Klesbiella* sp. and *Staphyllococcus aureaus* probably by the mechanism of membrane disruption or enzymes inactivation. Binding to adhesins, proteins, substrate deprivation, and

**Table 4.** Some characterized plant materials with antimicrobial activities.

| Plant/plant material | Characterised isolate | Source |
|---|---|---|
| *A. cordifolia* | Isopenteneyl guanidine | Lamikanre et al. (1990) |
| *Aspilia africana* leaf | Inisitol | Ita et al. (2010) |
| | Limonene | |
| | Alpha-pinene | |
| | Ethyl 3-(3,4-dihydroxyphenyl) acrylate | Faleye and Ogundaini (2012) |
| | 3-(4-dihydroxyphenyl)-oxo-2H-chromene-6-carbaldehyde | |
| *O. basilicum* | Linalool | Klimankova (2008) |
| | Methyl chavicol | |
| | Eugenol | |
| | Bergamotene | |
| | Methyl cinnamate | |
| | Geranyl acetate | Ozcan and Chalchat (2002) |
| | Methyl eugeno | |
| | Rosmarinic acid | Jamal et al. (2002) |
| *Stachyterpheta jamaicensis linn vahl* | Lanostance glycoside | Okwu and Offiong (2009) |
| *S. jamaicensis* | Steroidal glycoside | Okwu and Ohenhen (2010) |
| *N. oleander* | Oxoocyl-1-2-hydroxyundecanoate | Sharma et al. (2010) |
| | Heptacosane-3enyl-5-hydroxyhexanoate | |
| | Butelin | |
| | Butelinic acid | |
| | Stigmasterol | |
| | Neridienone A | Gupta andMittal (2010) |
| | Neridienone B | |
| *S. aromaticum* | 4-allyl-2-methoxyphenol | Rahimi et al. (2012) |
| *E. carophyllata* | Eugenol | Singh et al. (2012) |
| *H. sauveolens* | Stigmasterol | Jasani et al. (2012) |
| | Beta-myrcene | Vijay et al. (2011) |
| | Sabenene | |
| | (2E)-1-(2-hydroxy pheyl)penta-2-en-1-one | |
| | 1-{(3-hydroxy-5, 5-dimethylcyclohex-3-en-1yl)oxy}hexan-3-one | Jayakumar and Ganesh (2012) |
| *Datarium senegalense Gmelin* | Tetrahydroxyl anthonyanides | Okwu and Uchegbu (2009) |
| *Dataru metel linn* | Alkaloid sitosterol 1 | Okwu and Igara (2009) |
| *V. amygdalina* | Stigmasterol | Luo et al. (2008) |
| | Chondrillasterol | |
| | n-Hexadecanoic acid | |
| | Beta-Sitosterol | Onuegbu (1996) |

**Table 4.** Continued.

| Plant/plant material | Characterised isolate | Source |
|---|---|---|
| *Melanthera scandens* | Beta-caryophyllene<br>Limonene<br>Beta-phellandrene<br>Alpha-bisabolol<br>Alpha-humulene | Affia et al. (2011) |
| *A. meleguata* | Monoterpene indole alcohol | Okwu and Njoku (2010) |
| *Bridelia ferruginea berth* | Flavonoid chalcones<br>Anthocyanidines | Okwu and Ukanwa (2010a) |
| *G. kola* seed | Flavone glycoside | Okwu and Morah (2007) |
| *Datura metel linn* | Beta-Carboline alkaloid | Okwu and Igara (2011) |
| *F. exasperate* | Ficusamide<br>Furanocoumarines<br>Bergapten | Dongfack et al. (2012) |
| | Alpha-terpineol<br>Alpha-pinene | Oladosu et al. (2009) |
| *Peperomia pellucid* | Phytol<br>Hexadeconoic acid<br>Naphthaleno<br>Octadecanoic acidl | Wei et al. (2011) |
| *B. pinnatum* | Flavonoid glycoside<br>Rutin<br>Kaemoforol-3-glycoside<br>Beta-Sitosterol | Okwu and Uchenna (2009) |
| *Cleredendon splendens* | Flavonone-diglucoside<br>Hispudilin | Okwu and Uchenna (2009) |
| *A. conyzoides* | Coumarin | Widodo et al. (2008) |
| *C. alata* | Chrysanthemic acid<br>Luteoline | Okwu and Uchenna (2009) |
| *N. latifolia* | Nauclechine | Okwu and uchenna (2009) |
| *A. indica* (Neem) | Azadirachtin | Brown (2006) |
| *A. cordifolia* | Anthocyanidine glycosides | Okwu and Ukanwa (2010b) |
| *P. guineense* | Piperine<br>Piperidine | Scott et al (2005) |

**Table 4.** Continued.

| Plant/plant material | Characterised isolate | Source |
|---|---|---|
| *C. papaya* | Carpaine<br>Pseudo-carpaine | Njoku and Obi (2009) |
| *C. papaya* | Xanthine<br>Violaxanthine | Njoku and obi (2009) |
| *Allium cepa L.* | Allicin | Gurjar et al. (2012) |
| *A. satvum L.* | Allicin | Gurjar et al. (2012) |
| *Thymus vulgaris L.* | Caffeic acid | Gurjar et al. (2012) |
| *Ricinus cumminis L.* | Ricinine<br>Ricininoleic acid | Gurjar et al. (2012) |

intercalation into cell walls and DNA have been advanced as well by many workers as possible mechanisms for the observed inhibitions (Echeverrigaray et al., 2010; Gurjar et al., 2012). Allicin (*Alliaceae*) is readily cell-membrane permeable and undergoes thio-disulphide exchange reaction with amino acids and proteins. The fungitoxic property of allicin is assumedly thought pivoted on this attribute (Shusarenko et al., 2008). Besides, it is also thought that the mode of action of allicin may also be by mediation of lipoperoxide production in fungal plasma membrane leading to increased permeability (Rongai et al., 2012). With regard to saponins, remarked the foregoing source, their mechanism of antifungal action is not well understood but it is believed to complex with sterols in the cell membrane leading to pores formations and consequent loss of cell membrane integrity. According to Brown (2006), cinnamaldehyde (Cinnacure, Cinnamite) a botanical pesticide, is believed to impair energy production of target organisms. He however reported that its exact mode of action is not well understood, though interference with glucose uptake is assumed. Concrete studies to ascertain these claims and postulations seem lacking. However, experts maintain that unless something is done drastically to improve the effectiveness of biopesticides, the growth in their popularity will remain only gradual (Biopesticides, 2012). We, as a matter of necessity must understand how these active isolates interfere with the physiology of the target organisms before thinking of improvements. To this end therefore, the challenges facing us in this area of plant disease management now are to determine:

- What constituent(s) make(s) up the test extract(s) and what functional groups do(es) the extract(s) possess?
- What are their binding sites on the target pathogen?
- What metabolic processes or pathways do they affect by so doing?
- How do they therefore effect the observed inhibitions and/or kill the target pathogen?
- In most cases listed in Table 1 above, these are not thoroughly understood yet.

## CONCLUSION

Reports show that majority of the evidences of plant-based chemical activity against plant pathogenic fungi and other micro-organisms of agricultural importance were provided from *in vitro* evaluations. Crude extract evaluations have become very rudimentary. Numerous phyto-chemicals which have demonstrated *in vitro* effects should be evaluated on-farm under natural or induced infections, to further determine and establish their efficacy in controlling the incidences of pathogen-borne diseases in crops in a multi-factor environment. Information on the active ingredients of plant extracts is sparingly available while that of the mechanism of action of isolates is almost non-existent. These are high profile, high-tech areas of scientific endeavours, and multi-disciplinary in nature. Therefore further focus and articulate researches to improve the effectiveness, target specificity and shelf storageability of botanicals are pressingly imperative. To achieve this, we must liaise and collaborate effectively with scientists in biochemistry, plant physiology, biotechnology, pharmacology and natural products chemistry. It is in the light of this kind of collaboration and purpose-guided assays that the much anticipated outcomes of identifying, isolating, characterizing and understanding isolate-pathogen interactions which will lead us to the knowledge of the MOA of the

active principles of plant-based natural products will be achieved in the near future.

## SUGGESTIONS AND FUTURE TRENDS

1. Investigators should be encouraged to conduct on-farm evaluations of crude plant extracts against a wide range of pathogens of various high value field and vegetable crops. *In vitro* trials have become too narrow for us to base our conclusion of bioefficacy of crude extracts against pathogenic fungi upon.
2. Given proven phyto-fungal toxicity of the plant materials and assertions on their effectiveness especially from actual field trials in the management of plant health challenges; many concerted and directed efforts and thrusts should hence be geared toward chemical exami-nations of the plant materials in all future investigations with a view to:

- Determining their phytochemical compositions;
- Determining their chemical functional groups and their relative positions on the carbon skeletons;
- Isolating their active ingredients;
- Elucidating and characterizing the structure of isolates so as to enhance:

- Studies on their modes of action on pathogens,
- Phytotoxicity of the principles on host plants and,
- Possible means of improving their effectiveness and synthesis.

3. Departmental collaborations should be sought with highly equipped and established laboratories in the USA, UK, China and India to enable investigators overcome complex issues of chemical structure elucidation of isolates and isolate-pathogen interactions.
4. Cost-benefit evaluations should be incorporated in our trials to scientifically establish the cost effectiveness of the plant extracts *vis-a-viz* synthetic chemical products rather than base this on guess works.
5. Appropriate tests on the mammalian toxicity of isolates from plant extracts are encouraged and should be thoroughly and speedily conducted to overcome the challenges of bans of products after introduction into the wider market.

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