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ETHNOMEDICINAL POTENTIAL OF *KHAYA SENEGALENSIS* (DESR) A. JUSS (MAHOGANY) ON PATHOGENIC FUNGI OF VEGETABLES IN SOKOTO METROPOLIS

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ABSTRACT

The antifungal efficacy of *Khaya senegalensis* (leaf and bark) extracts was tested *in vitro* on three pathogenic fungi of vegetables in Sokoto metropolis, at different concentrations (1mg/1ml, 3mg/ml, 6mg/ml, 9mg/ml and 12mg/ml). The solvents used were water, acetone and Millet Steeped Water (MSW). The plant extracts were found to be effective in reducing the growth of the pathogenic fungi. Acetone and MSW extracts were more effective in inhibiting the growth of the test fungi than the aqueous extracts. MSW recorded highest percentage growth inhibition (83.05± 0.53%) of *Aspergillus niger*, followed by acetone extract (79.01± 0.53%) and aqueous extract had no inhibitory effect. The result also revealed that acetone leaf extracts of the plant had antifungal effect on growth of *M. racemosus* (56.62±1.65%) and *Rhizopus oryzae* (44.15±0.52%). The results indicate that the leaf and bark extracts of the plant have antifungal properties and could be used for the management of growth of these phytopathogenic fungi. MSW and acetone proved to be more effective in reducing the mycelia growth of the fungi than aqueous extract. MSW should be utilised as the solvent for extraction because of its effectiveness as extracting solvent and its availability at low cost.

KEYWORDS: Antifungal, K. Senegalensis, Plant Extracts, Pathogens, Vegetables

INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions as well as defence against attack from predators. About 12,000 chemical compounds have been isolated from plants. These are estimated to be less than 10% of the total chemical compounds contained in the plants (Lichterman, 2004; Tapsell *et al*., 2006). Chemical compounds in plants mediate their effect on the human body through processes similar to chemical compounds found in conventional drugs. Herbal medicines can therefore be said to perform similar work done by conventional drugs. All plants produce chemical compounds known as phytochemicals as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants (Lichterman, 2004) and secondary metabolites compounds which are found in a smaller range of plants (Tapsell *et al*., 2006), serving a more specific function (Meskin, 2002). Secondary metabolites have therapeutic actions in humans. They can be refined to produce drugs, examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove (Meskin, 2002). Plants synthesize a bewildering variety of phytochemicals, such as quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serve as plant defence mechanisms against pathogenic microorganisms. Seasonal variations can affect the chemical composition of the plants and thus biological activity (WHO,

2003). The geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, soil, propagation method, etc (Adoum *et al*., 1997). Time of collection of plant parts also affects its effectiveness (Odugbemi, 2008). Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiological absorption of the extract, preservative action and inability to cause the extract to complex or dissociate (Hughes, 2002)

Khaya senegalensis (*Madachi* in Hausa, *Oganwa* in Yoruba, and *Ono* in Igbo), is a savanna tree, easily recognised by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules. It is a tree of 30 m high and 3 m girth, with dense crown and short bole covered with dark dry scaly bark. It is identified by its slash dark pink bitter yielding gum when wounded. The leaves have 3 - 4 pairs of leaflets which are more or less elliptic, round, obtuse or shortly acuminate at apex; the stalks of leaflets are 4mm long (Keay *et al*., 1989). *K. senegalensis* has attracted world-wide attention for its high quality timber production. The stem-back and leaves of *K. senegalensis* have been used in Northern Nigeria in forms of decoction and concoctions for the cure of mucous diarrhoea, syphilis, pyrexia and malarial fever (Olayinka *et al*., 1992). Dried stem-bark is used externally for the treatment of skin affections (Grand, 1989). The bark of the tree is very bitter and is extensively used for the treatment of fever and dressing ulcers on the backs of sheep, camels and horses. The flowers are used for treating stomach diseases, and as an ingredient in anti syphlytic prescriptions. The plant is utilized ethno-medicinally as a remedy for several human and animal ailments in northern Nigeria, by the Hausa and the Fulani tribes (Deeniand and Sadiq, 2002; Nacoulma, 1996).

The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and to the existing human eco geographical conditions, as some of those chemicals have already been proved to be either mutagenic, carcinogenic or teratogenic. Many pathogenic microorganisms have acquired resistance to synthetic pesticides (White *et al*., 2002). This seriously hinders the management of diseases of crops and agriculture products. Majority of farmers in Sokoto metropolis are peasant farmers and practise subsistence farming. Thus, they could hardly afford the high cost of pesticides for managing and controlling the spread of diseases in their farms. Keeping in view the side effects of synthetic chemical management of plant, the use of plant extract in disease management is therefore gaining importance (Kiran *et al.* 2006; Okigbo, 2009).This paper therefore was intended to find a better alternative to the use of fungicides through the use of selected plant extracts which are widely believed to possess natural bioactive products that are non-phytotoxic, more systemic and easily biodegradable (Gottlieb, 1990), readily available and affordable at low cost. In addition, these plants bioactive compounds are considered to be environmentally safe for control and management of plant pathogens both on field, in the market and in storage places.

MATERIALS AND METHODS

Collection of Plant Samples

Fresh leaves and bark *Khaya senegalensis* already identified by the department of biological Sciences, Usmanu Danfodiyo University, Sokoto was collected from within the environment of Faculty of Science, washed thoroughly with running tap water and then rinsed with distilled water. The fresh plant material was air-dried under the shade. The dried sample was pounded to obtain a powdered with the help of a pestle and mortar. The powder sample was stored in airtight bottles till needed for use.

PREPARATION OF THE PLANT LEAF AND BARK EXTRACTS

Aqueous Extraction

Aqueous extract was prepared using the method of Bajwa *et al*. (2007) slight modification. One hundred gram (100g) of the powder samples was added to 1litre of distilled water to obtain a mixture. The mixture was left to stand for 24 hrs, then filtered through double layered muslin cloth and the filtrate was poured into a beaker and evaporated on hot plate oven at 40^0C. The residue for each sample, the extract, was then preserved aseptically in bottles at 5^oC for further use.

Solvent Extraction: Acetone

The method of Alkhail (2005) was used with slight modification for the extraction. One hundred gram (100g) of the powder samples of the plant was added separately to 1litre acetone to obtain a mixture. The mixture was left to stand for 24 hrs, then filtered through double layered muslin cloth and the filtrate was poured into a beaker and evaporated on hot plate oven at 40^0C. The residue from sample, the extract, was then preserved aseptically in bottles at 5^oC for further use.

Solvent Extraction: Milled Millet Steeped Water (FMMSW)

Millet fermented water production was carried out by overnight steeping of 2 kg of pearl millet (*Pennisetum glaucum*) obtained from Sokoto Central Market. The steep water was then discarded and wet milling of the millet grains was carried out. Water was added to the milled materials to make thick slurry. The slurry was sieved to remove chaff. The slurry was allowed to ferment and sediment/decant for 2–3 hours (Lei and Jakobsen, 2004). The liquid top-layer was separated from the sediment at the bottom-layer. The liquid water collected in a 10 litre gallon as fermented milled millet steeped water (FMMSW) and allowed to stand for 3 days before it is put into use. One hundred gram (100g) of the powder samples was added separately to 1litre FMMSW to obtain a mixture. The mixture was left to stand for 24 hrs, then filtered through double layered muslin cloth and the filtrate was poured into a beaker and evaporated on hot plate oven at 40^0C. The residue from sample, the extract, was then preserved aseptically in bottles at 5^oC for further use.

Culture Medium (Potato Dextrose Agar (PDA)

Potato dextrose agar medium was prepared according to manufacturer's specification. Thirty nine grams (39g) potato dextrose agar (dehydrated) was mixed with 1litre of distilled water. The mixture was boiled to dissolve particles and then autoclaved for 15min at 121°C to get it sterilised. The pH was adjusted to 5.6 (BAM, 1998).

Antifungal Efficacy of the Plant Extracts on the Fungal Pathogens

The efficacy of each of the plant extracts was tested against three pathogenic fungi (*Rhizopus* sp, *Aspergillus* sp *and Mucor* sp.) isolated from vegetable crops. This was done through the growth inhibition test *in vitro*. Food poisoning techniques as described by (Sangoyomi, 2004) with slight modification was used to study the efficacy of plant extracts on mycelia growth of the test fungi using. 0.05 g, 0.15g, 0.30g, 0.45g and 60 g of each of the plant extracts was separately dispensed in sterile test tubes and dissolved in 50ml distilled water to obtain mixtures of varying concentrations. The concentrations of each plant extract were 1%, 3%, 6%, 9% and 12% respectively. Each of the concentrations was dispensed per Petri dishes and 9ml of the media (molten PDA) was added to each of the extracts containing Petri dishes resulting in PDA-extract mixtures. These were gently rotated to ensure homogeneous dispersion of the extracts and then allowed to solidify. The PDA-extract mixture were inoculated at the centre of the Petri dishes with a 4mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the test fungi. Negative controls were set up using

blank agar plates (no extracts) and inoculated with each test fungus, and the positive control consisted of the fungicide which was prepared according to the manufacturer's direction by dissolving 0.5g in 100ml of sterile distilled water. Three replicate plates of PDA-extracts per isolates were arranged in an incubation room at an ambient temperature 35 ± 2^0C and radial growth was measured daily for 7days.

Measurement of Growth (Diameter)

Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition (Nene and Thapliyal, 1979).

$$\% \text{ Mycellial inhibition} = \frac{\text{Mycellial growth}_{(\text{control})} - \text{Mycellial growth}_{(\text{treatment})}}{\text{Mycellial growth (control)}} \times 100$$

Experimental Design and Statistical Analysis

Complete Randomized Design with three replicates was used. The data obtained from the study were statistically analysed using the SPSS statistical version 16. The data were subjected to analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) at $p<0.05$ significant level

RESULTS

The results of the study were presented in tables 1-2. All the plants extracts used were found to significantly reduce the growth of the fungal pathogens in vitro.

Antifungal Efficacy of *K. Senegalensis* (Leaf) on Fungal Pathogens

The inhibitory effect of acetone, aqueous and MMSW extracts of *K. senegalensis* leaf are shown on Table 4. Acetone extract reduced the growth of all the fungal pathogens with highest inhibition on the growth of *A. niger* (84.730±0.59%), closely followed by *Mucor* sp (56.62 ±1.65%) and the least was observed on growth of *R. oryzae* (44.15 ±0.52%). Aqueous extract had no effect on growth of any of the test fungi while MMSW had inhibitory effect on mycelia growth of *A. niger* (84.96±0.59%) and *M. racemosus* (18.59 ±1.65%). Growth of *A. niger* on acetone and MMSW extracts did not differ significantly $p<0.05$. There was a significant difference $p<0.05$ among the values observed at different concentrations of the extracts on growth inhibition of the pathogens. For growth of *R. oryzae* on the extract 12% concentration had the highest inhibitory value 22.48±0.68%, closely followed by 3% concentration 14.44±0.68%, then 9% concentration 14.07 ±0.68%, 6% concentration was next 11.61±0.68% and the least was 1% concentration 10.99±0.68%. Concentrations 3% and 9% on one hand, and 6% and 1% on the other hand, are not significantly different $p<0.05$. Concentrations 9% and 12% are not significantly different on growth inhibition of *M. racemosus* with values 40.37±2.13% and 39.38 ±2.13% respectively. Highest inhibitory value was observed in 9% concentration then, 12% which was closely followed by 6% concentration (24.31±2.13%), 3% concentration was next (17.84±2.13%) and least inhibition for growth of *M. racemosus* was observed in 1% concentration (3.46±2.13%). Growth inhibition of *A. niger* by the extracts at different concentrations was observed to be significantly different at $p<0.05$. 1% concentration recorded the highest inhibitory value (57.34±0.77%), then 9% (56.92±0.77%), 12% was next (56.32±0.77%) and the least was recorded at 3% concentration (55.32±0.77%). The interaction between the extractions, concentrations and the fungi was significant $p<0.05$.

Antifungal Efficacy of *K. Senegalensis* (Bark) on Fungal Pathogens

The inhibitory effect of acetone, aqueous and MMSW extracts of *K. senegalensis* leaf are shown on Table 5. Acetone extract had inhibitory effect on mycelia growth of all the pathogens. Highest inhibitory effect of the acetone extract was observed on the growth of *A. niger* with inhibition value of 82.21±0.53%, then, *M. racemosus* with the value 27.40±3.72% and the least was 9.19±0.25 for *R. oryzae*. Aqueous extract had no effect on growth of the test fungi. MMSW had no effect on the growth of *R. oryzae* but it reduced the growth of *M. racemosus* and *R. oryzae*. Highest inhibitory effect of MMSW was observed on mycelia growth of *A. niger* (83.09±0.53%) while 36.48±3.72 was the value recorded for *M. racemosus*. There was no significant difference ($p<0.05$) among the values recorded at different concentrations of the plant extract on growth inhibition of *A. niger*. 3% concentration had growth inhibitory value of 55.68±0.68%; 1 % had 55.67±0.68%; 6% had 55.53±0.68%; 12% had 54.51±0.68%; and 9% had 55.11±0.68%. *M. racemosus* had its highest growth reduction at 9% concentration (38.23±4.80%), followed by 35.80±4.80% at 12%, then 32.43±4.80% at 6% while there was no growth retardation at 1% and 3% concentrations. The plant extract had slight inhibitory effect on the growth of *R. oryzae* at different concentrations. The highest inhibitory value 7.83±0.32% was recorded 9% concentration then, 5.99±0.32% at 6%, next was 0.99±0.32% at 12%, followed by 0.50±0.32% at 3% concentration. There was no inhibition of growth at 1% concentration. There was significant interaction between the extracts, concentrations and the fungi $p<0.05$.

Table 1: Antifungal Efficacy of K. Senegalensis (Leaves) on Fungal Pathogens

Factor	Zone of Inhibition (%)		
	R. Oryzae	M. Racemosus	A. Niger
Extract			
Acetone	44.15[a]	56.6 2[a]	84.73[a]
Aqueous	0.00[b]	0.00[c]	0.00[b]
MMSW	0.00[b]	18.59[b]	84.96[a]
SEM±	0.52	1.65	0.59
Concentration (%)			
1	10.99[c]	3.46[d]	57.34[a]
3	14.44[b]	17.84[c]	55.32[a]
6	11.61[c]	24.31[b]	56.51[a]
9	14.07[b]	40.37[a]	56.92[a]
12	22.48[a]	39.38[a]	56.73[a]
SEM±	0.68	2.13	0.77
Interaction	**	**	**

abcd. Means bearing different superscript along the same column within subclass differ ($p<0.05$).

** ($p<0.05$)

MMSW- Milled Millet Steeped Water.

SEM- Standard Error Mean

Table 2: Antifungal Efficacy of K. Senegalensis (Bark) on Fungal Pathogens.

Factor	Zone of Inhibition (%)		
	R. Oryzae	M. Racemosus	A. Niger
Extract			
Acetone	9.19[a]	27.40[a]	82.81[a]
Aqueous	0.00[b]	0.00[b]	0.00[b]
MMSW	0.00[b]	36.48[a]	83.09[a]
SEM±	0.25	3.72	0.53

Concentration (%)			
1	0.00[d]	0.00[b]	55.67[a]
3	0.50[d]	0.00[b]	55.68[a]
6	5.99[b]	32.43[a]	55.53[a]
9	7.83[a]	38.23[a]	55.11[a]
12	0.99[c]	35.80[a]	54.51[a]
SEM±	0.32	4.80	0.68
Interaction	**	**	**

abcd. Means bearing different superscript along the same column within subclass differ ($p<0.05$).

** ($p<0.05$)

MMSW- Fermented Milled Millet Steeped Water.

SEM- Standard Error Mean

DISCUSSIONS

The mycelium growth of all the three fungal pathogens was reduced by the plant extract of *K. senegalensis* at different degrees. That indicates that the plant extract has the potentiality to reduce the growth of the test fungi which also suggests that the plant possess antifungal properties. That could be due to the high concentration of tannins, saponins, flavonoid and alkaloid present in both the leaf and bark extracts of the plant. Tannin had been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them (Sadipo, 1991). Saponin has been reported to be active antifungal agents (Fluck, 1973). On the other hand, alkaloids are commonly found to have antimicrobial property (Omulokoli *et al*., 1997) and flavonoids are shown to inhibit growth of microbes which are resistant to antibiotics (Linuma *et al*., 1994).

The study indicated that there was a significant difference in the antifungal activity of the extraction medium. Aqueous medium was not as effective as Acetone and MMSW media on their antifungal activity. This could be because water is an inorganic solvent, therefore may not be able to effectively dissolve the bioactive compounds responsible for antifungal and antimicrobial activities in the test plant. Acetone and MMSW on the other hand, are organic solvents and for that reason could dissolve the bioactive compounds effectively, thus have better antifungal activity. The difference in the antifungal activity of the plants parts may also be due to the differences in the solubility of the bioactive substances in the solvents or presence of inhibitors against fungicidal principles (Anukwuorji *et al*., 2013), this is in line with the reports of Quasem and Abu-blan (1996); Amadioha (2000); Onifade (2002); Okigbo *et al*. (2009); Ijato *et al*. (2010).

The result showed that the extent of inhibition caused by the plant solvent extracts varied. The highest reduction in mycelia growth of the pathogens was recorded from MMSW extract followed by Acetone extract and the least was aqueous. The disparity in the level of efficacy of the solvent extracts was in line with reports of Amienyo and Ataga, 2007; Fukunang *et al*., 2000; Ijato, 2011; Okigbo *et al*., 2009. The inhibitory effect of MMSW could be due to the presence of large number of viable lactic acid bacteria which help in inhibiting the growth of microorganisms. MMSW is a fermented product which contains flavour and aromatic compounds, biomass proteins/amino acids, carbohydrates, vitamins and other products of respiratory/biosynthetic process like lactic acid, ethanol, acetylaldehydes, pyruvic acids, which help in altering the pH of the food to levels that do not favour the growth of pathogenic microorganisms (Au and Field, 1981; Baghel *et al*., 1985; Steinkraus, 1996; Deshpande and Salunke, 2000; Beaumont, 2002; Anna *et al*., 2003; Kalui *et al*., 2009).

The leaf and the bark extract of the plant had inhibitory activity on mycelia growth of all the three pathogenic fungi regardless of the extraction solvents and the concentrations of the extracts, with the leaf extract having higher inhibitory activity. The disparity in the level of efficacy could be due to the presence of different phytochemical compounds present in the plants. These phytochemical compounds differ in their ability to deter or completely stop the growth of the pathogens. This study is in line with the findings of Anukwuorji *et al.* (2013) who reported that the medicinal, pharmacological and antimicrobial values of plants lie in their component phytochemical. The result indicated that leaf extract was the most effective in terms of antifungal activity. This could be due to the fact that leaves are considered as the most important life giving part of the plant body, as they carry out the process of photosynthesis in order to produce carbohydrate and a variety of compounds like oils and proteins which are utilised by the plants to build up materials for survival and reproduction, at the same time, animals also do use such materials for food, medicines, dyes, fibres, etc (Bareja, 2011), therefore, the leaves are loaded with bioactive materials.

CONCLUSIONS

The growth of all the three fungal pathogens differs in their levels of inhibition by the plant extract. MMSW was the most effective extract for antifungal activity, closely followed by acetone and the least was aqueous extract. The leaf extract has higher inhibitory activity. The disparity in the level of efficacy could be due to the presence of different phytochemical compounds present in the plants.

RECOMMENDATIONS

- *K. senegalensis* have the potentials for antifungal activities of vegetable crop pathogens therefore it should be utilised.
- MMSW should be utilised as the solvent for extraction because of its effectiveness as extracting solvent and its availability at low cost.

REFERENCES

1. Adoum, O.A, Akinniyi, J. A. and Omar, T. (1997). The effect of Geographical location on the antimicrobial activities and trace Element concentrations in the root of Calotropis procera (Ait.) R.Br. Annals of Botany. **13**(14): 199-207.
2. Alkhail, A.A. (2005). Antifungal activity of some extracts against some plant pathogenic fungi. Pak.J. Biol. Sci., **8** (3): 413 – 417.Amadioha, A.C. (2000). Fungitoxic effect of some leaf extracts against Rhizopus oryzae causing timber rot of potato. Arch. Phytopathol. PFlan.; **34**:1-9.
3. Amienyo, C.A and Ataga, A.E. (2007). Use of indigenous plant extracts for the protection of mechanically injured sweet potato (Ipomea batatas (L) Lam) tubers. Scientific Research and Essay 2(5): 169 – 170.
4. Annan, N. T, Poll, L, Sefa-Dedeh S., Plahar, W. A. and Akobsen M. (2003) Volatile compounds produced by Lactobacillus fermentum, Saccharomyces cerevisiae and Candida krusei in single starter culture fermentations of Ghanaian maize dough. Journal. Appl. Microbiol. **94**(3): 462-474.
5. Anukwuorji, C.A., Anuagasi C.L. and Okigbo R.N. (2013). Occurrence and Control of Fungal Pathogens of Potato (Ipomoea Batatas L. Lam) With Plant Extracts. PhTechMed **2**:278-289

6. Au, P.M. and Fields, M. L. (1981). A research note on nutritive quality of fermented sorghum Journal. Food Sci. **46**: 652-654.

7. Azu, N. C. and Onyeagba, R. A. (2007). Antimicrobial Properties of Extracts of Allium cepa (Onion) and Zizipus officinale (Ginger) on Escherichia coli, Salmonella typhi and Baccilus subtilis. International Journal Of Tropical Med. **3**: 123-127.

8. BAM. (1998). Bacterial Analytical Manual. 8th Edition, Revision A. Baghel RPS, Netke SP, Bajpai LD (1985). Nutritive value of kangni. Poultry Guide **22**: 28-29.

9. Bajwa, R., Shafique,S. and Shafique, S. (2007). Appraisal of antifungal activity of Aloe vera. Mycopath, **5** (1): 5-9.

10. Beaumont, M. (2002). Flavouring composition prepared by fermentation with Bacillus spp. Int. Journal. Food Microbiol. **75**: 189-196.

11. Bareja, B. G. (2011). Light Intensity in Plant Growth and Development. Cropsreview.com. www.cropsreview.com/light- intensity.htmlCachedSimilar Retrieved April 2011.

12. Deeniand, Y.Y. and Sadiq, N.M. (2002). Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (Tapinanthus dodoneifolius (DC) Danser (Lorantheaceae): An ethonomedicinal plant of Hausa, Northern Nigeria. J Ethnopharmocol. **83**(30): 235-240.

13. Deshpande, S.S. and Salunkhe, D. K. **(**2000). Grain legumes, seeds and nuts: rationale for fermentation. Fermented grains legumes, seeds and nuts: a global perspective. FAO Agricultural Services Bulletin. **142**:1-32.

14. El-Olemy, M. M., Al-Muhtadi, F. J. and Afifi, A. A. (1994). Experimental Phytochemistry. A Laboratory Manual. King Saud University Press. Pp8-9

15. Fluck, H. (1973). Medicinal plants and their uses. W. Feulshom and comp. Ltd, New York. Pp 7-15.

16. Fukunang, C.N, Ikotun, T, Dixon, A.G.O. Akem, C.N, Tembe, E.A and Nukenine E.N. (2000). Efficacy of Antimicrobia plant crude extracts on the growth of Colletotrichum gleosporoides F. sp manihotis. Pakistan Journal.of

17. Biol.Sci**. 3**(6):928-932.

18. Gottlieb, O. R. (1990). Phytochemicals: differentiation and function. Phytochemistry. **29**: 1715- 1724.

19. Hughes, I. (2002). Science in Africa Magazine. Issue **56.**

20. Ijato ,J. Y.; Oyeyemi, S. D., Ijadunola ,J. A. and Ademuyiwa, J. A. (2010). Allelopathic effect of leaf extract of Azardirachta indica and Chromolaena odorata against post harvest and transit rot of tomato (Lycopersicum esculentum L). Journal of American Science, **6**(12).

21. Ijato J. Y. (2011). Evaluation Of Antifungal Effects Of Extracts Of Allium sativum And Nicotiana tobacum Against Soft Rot Of Yam (Dioscorea alata). Researcher, **3**(2): 1-5.

22. Kalui, C. M., Mathara, J. M., Kutima, P. M., Kiiyukia, C., and Wongo, L. E. (2009). Functional characteristics of Lactobacillus plantarum and Lactobacillus rhamnosus from ikii, a Kenyan traditional fermented maize porridge.

Afr. J. Biotechnol. **8**(17): 4363-4373.

23. Keay, R.W.J., Onochie, C.F. F. and Stanfied, J. (1984). A Revised version of Trees of Nigeira (1964). Clarendon Prss, New York. Pp 339-340.
24. Kiran, K., Linguratu, S. and Adiver, S. (2006). Effect of plant extract on Sclerotium rolfsii, the incitant of stem rot of groundnut .J. Mycol.PL.Pthol., **36**:77-79
25. Lei, V and Jakobsen, M. (2004). Microbiological characterization and probiotic potential of koko and koko sour water, African spontaneously fermented millet porridge and drink. Journal of Applied Microbiology. **96**. 384–397
26. Linuma, M., Tsuchiya, H., Sato, M., Yokoyama, J., Ohyama, M., Ohkawa, Y., Tanaka, T., Fujiwara, S. and Fujii, T. (1994). Flavanones with potent antibacterial activity against methicillin-resistant Staphylococcus aureus. J Pharm Pharmacol.
27. **46**(11):892-895.
28. Lichterman, B. L. (2004). Aspirin: The Story of a Wonder Drug. British Medical Journal **329** (7479): 1408. doi:10.1136/bmj.329.7479.1408.
29. Meskin, M. S. (2002). Phytochemicals in Nutrition and Health . CRC Press. p. 123. ISBN 9781587160837.
30. Nacoulma, O.G. (1996). Medical Plants and Traditional Medical Practices in Bukina Faso: the case of the central plateau. University of Ouagadougou. PhD. Thesis p 261-320.
31. Nene, Y. L. and Thapliyal, B. W. (1979). Fungicides in plant disease control. Oxford and IHB publ. Co., New Delhi. 425.
32. Okigbo, R.N. (2009). Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi State, Nigeria. American-Eurasian J. of Sust. Agric.**3** (3): 407- 409.
33. Okigbo, R.N., Anuagasi,C. L., Amadi, J. E. and Ukpabi, U. (2009). Potential inhibitory effects of some African tuberous plant extracts on Escherichia coli, Staphylococcus aurteus and Candida albican. International Journal of Integrative Biology (IJIB India) **6**(2): 91-98.
34. Olayinka, D.O., Onoruvwe, O. and Lot, T.Y. (1992). Cardiovascular effects in rodents of methanolic extract of Khaya senegalensis. Phytother. Res. **6**: 282-284.
35. Odugbemi, T. (2008). A textbook of Medicinal plants from Nigeria. University of Lagos Press, Lagos, Nigeria. Pp.542.
36. Omutokoli, E., Khan, B. and Chhabra, S. (1997). Antiplasmodial activity of four Kenyan medicinal plants. Journal Ethnopharmacol. **56**(2): 133-137.
37. Onifade, A.K. (2002). Antifungal effect of Azadirachta indica A. Juss extracts on Collectotricum lindemathianum. Global J. Pure. Appl. Sci., **6**(3): 423-428.
38. Qasem, J. R., Al – Abed, A. S. and Abu – Blan, H. A. (1995). Antifungal activities of clammy inula (I. viscosa) on Helminthosporium sativum and Fusarium oxysporum f. sp. Lycoperisci. Phytopath.– Mediterranean. **1**: 7 - 14.

39. Sangoyomi, T. E. (2004). Post-Harvest Fungal Deterioration of Yam (Dioscorea rotundata Poir) and its control. Thesis. Internally Institute of Tropial Agriculture. Ibadan, Nigeria. Pp 179.

40. Sodipo, O.A., Akiniyi, J.A., Ogunbamosu, J.U. (2000). Studies on certain on certain characteristics of extracts of bark of Pansinystalia macruceras (K schemp) picrre Exbeille. Global J. Pure Appl. Sci., **6**: 83-87.

41. Steinkraus, K. H. (1996). Handbook of Indigenous Fermented Foods. Marcel Dekker, Inc. Tapsell, L.C, Hemphill, I., Cobiac, L. (2006). "Health benefits of herbs and spices: the Past, the Present, the future". Med. J. Aust. **185** (4): S4–24.

42. White, D. G, Zhao, S., Simjee, S., Wagner, D. D. and McDermott, P. F. (2002). Antimicrobial resistance of food borne pathogens. Microbes Infect. **4**: 405-412.

43. World Health Organization. (2003). Traditional medicine. Fact sheet Number 134. In: h ttpo://www.whi.int/medicentre/factsheet/fsl34/e