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Phytochemical Screening of Aqueous, Ethanol and Methanol leaf Extracts of *Diospyros mespiliformis, Quisqualis indica and Aframomum melegueta*

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ABSTRACT

A comparative analysis using aqueous, ethanol and methanol extract of the ground samples of *Diospyros mespiliformis, Quisqualis indica and Aframomum melegueta* leaves were examined for the Qualitative and semi-quantitative screening of phytochemicals. These chemical substances were significantly demonstrated more in ethanolic and methanolic extracts than aqueous extraction of the samples. It was observed that alkaloid was absent in aqueous extraction of all the plant leaves, but was present in ethanol and methanol extraction. The analysis done on *Diospyros mespiliformi* reveal the presence of alkaloids, saponins, Triterpenes, flavonoids tannins and Cyanogenic glucosides. However, alkaloids, triterpenes and cyanogenic glucosides were absent when aqueous extraction was applied. Triterpenes and Cyanogenic glucosides were completely absent in *Aframomum melegueta* and *Qusquali Indica* leaves extracts, while alkaloids and flavonoids were only absent in aqueous extraction. The ethanolic and methanolic extracts appeared to be a better way of detecting phytochemicals when compared aqueous extraction. *Diospyros mespiliformis* extract possess more quantity of these phytochemical more than *Quisqualis indica and Aframomum melegueta* leaves extracts. The presence of these chemicals suggest that these plants could possess some pharmacological behavior.

Keywords: Phytochemical analysis, *Diospyros mespiliformis, Quisqualis indica and Aframomum melegueta*

INTRODUCTION

Diospyros mespiliformis also known as African ebony is a large deciduous tree found mostly in the savannas of Africa. It is confined to tropical and sub-tropical regions notably in Africa [1, 2]. The plant is used as dressing for burns, antibacterial agent and astrigent in

diarrhea. Over dose of concentrated decoction can cause abortion [3]. Ripe fruits of *Diospyros mespiliformis* are relished by indigenous people. The edible fruits is used fresh in fermented drink or dried and stored for later use [4]. The leaves are eaten by elephant, giraffe, black rhino, baboons etc; a definite asset to any farm. *D. mespiliformis* makes good fuel wood and charcoal. The wood is durable and used to make spoons, canoes, furniture, carving walking sticks and many constructions purposes as it is fungal and termite resistant. A secondary benefit from this tree is the flavor it gives to termites resting around the tree [5, 6]. The tree is used for shade and also makes an incredible screen or windbreak. The leaves, bark and roots of the tree contain tannin, which can be used as a styptic to staunch bleeding [7].

Quisqualis indica A. L., also known as the Rangoon creeper is a ligneous vine that can reach from 2.5 meters to 8 meters, it belongs to the family *Combretaceae.* Its leaves are elliptical with an acuminate tip and a rounded base. The plant is mainly used for traditional medicine. Decoction of the root, seed or fruit can be used as antihelminthic to expel parasitic worms or for alleviating diarrhea. The fruits and leaves can be used to combat nephritis and to relieve pain caused by fever [8, 9].

*Aframomum melegueta (*K. Schum.*)* is specie in the ginger family, *Zingiberaceae.* The specie, commonly known as grains of paradise, melegueta pepper, alligator pepper, Guinea grains, or Guinea pepper, is obtained from the ground seed; it gives a pungent, peppery flavour. It is a herbaceous perennial plant native to swampy habitats along the West African coast [10,11,12,13]**.** The alligator pepper is extensively used as a common ingredient of many traditional medicines. The decoction of the leaves is used for small pox and chicken pox. As a purgative, galactogogue (to increase the production of breast milk), antihelmintic and haemostatic agent (purifies the blood) in medicinal applications. Also used as a vermifuge and stimulant. Further it is used against intestinal infections, infestations, to calm indigestion and heartburn. It also posses potent anti-inflammatory activity with favourable gastric tolerability profile [14, 15].

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens [16]. Some phytochemicals have been used as poisons and others as traditional medicine [17]. The ground samples of *D. mespiliformis, A. melegueta* and *Q. indica* were examined for the presence of chemical constituents such as tannins, alkaloids, flavonoids, terpenoids, cyanogenic glycosides, and saponins. Alkaloids are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms, they can be purified from crude extracts of these organisms by acid-base extraction. Alkaloids have a wide range of pharmacological activities [18] and many have

found use in traditional or modern medicine, or as starting points for drug discovery [19]. Saponins are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative [20]. In plants, saponins may serve as anti-feedants [21] and to protect the plant against microbes and fungi. However, they are often bitter to taste. Triterpenes composes of three terpene units with the molecular formula $C_{30}H_{48}$; they may also be thought of as consisting of six isoprene units. Triterpenes exist in a huge variety of structures with nearly 200 different skeletons known from natural sources or enzymatic reactions [22]. These may be broadly divided according to the number of rings present. Animals, plants and fungi all create triterpenes. Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals [23]. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies [25, 26, 27, 28, 29]. Tannin is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids [30,31,32]. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and might help in regulating plant growth. They are an important ingredient in the process of tanning leather [33] and produces different colors with ferric chloride [34].

MATERIALS AND METHODS

A comparative analysis using aqueous, ethanol and methanol extract of the ground samples of *D. mespiliformis, A. melegueta* and *Q. indica* were examined for the Qualitative and semi-quantitative screening of chemical constituents such as tannins, alkaloids, flavonoids, terpenoids, cyanogenic glycosides, saponins, following the descriptions of Krishnaiah et al. (2009) [20] and Mikail (2010) [26].

Test for tannins: About 0.5ml of crude in a test tube was allowed to stand for 2min. Thereafter; $FeCl_3$ solution (0.1%, 0.2ml) was added to the samples (2ml) and observed for brownish-green or blue-black colouration indicating the presence of tannins.

Test for flavonoids: To 2ml of the extracts was added a few drops of 1% ammonia solution. Observation of a yellow colouration indicated the presence of flavonoids.

Test for Saponins: The extracts (2ml) were added to 18ml of distilled water and heated in a boiling water bath for 5 min. thereafter the extracts were filtered through what-man No 1 filter paper. The filtered sample (10ml) were each mixed with 3 ml of distilled water in a test

tube and shaken vigorously to obtain a stable froth. The froth was then mixed with three drops of olive oil and observed for the formation of emulsion which indicated the presence of saponins

Test for Alkaloids: To the extracts (0.2ml) in a test tube was added 5 drops of iodine solution. The presence of alkaloids was indicated by the appearance of dark-brown to yellow colouration in the tubes.

Test for Terpenoids: About 0.5g of extracts in 2ml of chloro form. Concentrated sulphuric acid (H_zS_{04}) carefully added to form a layer. A reddish brown colouration of the interface formed indicated the presence of terpenoids

Determination of Cyanogenic glucosides: About 0.5 ml of the extracts was placed in a test tube. A moist sodium picrate indicator paper was suspended in the neck of the test tube using cork to trap it. The closed tube was placed in a warm water bath at 45^{0}C for 1 hour. A pink colouration of the indicator paper indicted a positive result.

RESULTS

Table 1. Phytochemical Analysis of *Diospyros mespiliformis* Extracts from Various Solvents.

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	---	(++)	(++)
Saponins	(+)	(++)	(++)
Triterpenes	---	(+)	(+++)
Flavonoids	(+)	(+)	(++)
Tanins	(+)	(++)	(++)
Cyanogenic glucosides	---	(+)	(+)

Key: +++ = high concentration, ++ medium concentration, + = low concentration, - = not detected.

Table 2. Phytochemical Analysis of *Qusquali Indica* Extracts from Various Solvents

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	---	(+)	(+)
Saponins	(+)	(+)	(+)
Triterpenes	---	---	---
Flavonoids	---	(+)	(+)
Tanins	(+)	(++)	(++)
Cyanogenic glucosides	---	---	---

Key: +++ = high concentration, ++ medium concentration, + = low concentration, - = not detected.

Table 3. Phytochemical Analysis of *Aframomum melegueta* (AM) leave Extracts from Various Solvents

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	---	(+)	(+)
Saponins	(+)	(++)	(++)
Triterpenes	---	---	---
Flavonoids	---	(++)	(++)
Tanins	(+)	(++)	(++)
Cyanogenic glucosides	---	---	---

Key: +++ = high concentration, ++ medium concentration, + = low concentration, - = not detected.

DISCUSSION

The Phytochemical analysis of the plant materials using different solvent unravelled a number of chemical substances both qualitatively and quantitatively. Flavones, for instance which are known to be synthesized by plants in response to microbial infection, inhibit the initiation, promotion and progression of tumours [35, 36, 37]. Flavonones exhibit inhibitory effects against viruses [38] including HIV and respiratory syncytial virus [3,4, 6]. Terpenoids were reported to be active against bacteria, fungi, viruses and protozoa [17, 18, 19, 39]. It is believed to be active against viruses by envelope disruption by the lipophilic compounds. Alkaloids have been commonly found to have antimicrobial properties [40]. It is also useful against HIV infection as well as intestinal infections associated with AIDS (0 McMahon et al., 1995). Tannins are found in almost every plant part; wood, leaves, bark, roots and fruits [7]

and tannin containing beverages can cure or prevent a variety of viral infections [14]. At least two studies Nonaka et al (1990) and Kaul et al (1995) have shown tannins to be inhibitory to viral reverse transcriptases. Tannins (hydrolysable) also show anti- carcinogenic and anti-mutagenic effects [25].

These phytochemical were significantly demonstrated in ethanolic and methanolic extracts of *Diospyros mespiliformis* and *Aframomum melegueta.* The ethanolic extracts and methanolic extracts appeared to be a better way of detecting phytochemicals than aqueous extract, because it was observed that alkaloid was absent in aqueous extraction of the plant leaves, but was present in ethanol and methanol extraction. The analysis done on *Diospyros mespiliformi* reveal the presence of alkaloids, saponins, Triterpenes, flavonoids tannins and Cyanogenic glucosides. However, alkaloids, triterpenes and cyanogenic glucosides were absent when aqueous extraction was applied. Triterpenes and Cyanogenic glucosides were completely absent in *Aframomum melegueta* and *Qusquali Indica* leaves extracts, while alkaloids and flavonoids were only absent in aqueous extraction. The presence of these chemicals suggests that these plants could posses some pharmacological behavior. This finding is in agreement with the finding of Eloff (2008) [10] who examined a variety of extractants for their ability to solubilize antimicrobials from plants and ranked them in the order; methanol, ethanol, and water and posited also that most active inhibitors extracted are not water soluble. It is also in agreement with results of Agatemor (2009) [1] who found that ethanolic extracts of some Nigerian spices were more potent than the aqueous extracts against common food borne microorganisms including Staphylococcus aureus, Klesbsiella pneumonia, Proteus vulgaris and Streptococcus faecalis. *Diospyros mespiliformis* extract possess more quantity of these phytochemical more than *Quisqualis indica and Aframomum melegueta* leaves extracts. The presence of these chemicals suggest that these plants could possess some pharmacological behavior.

REFERENCES

1. Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigeria species against four food borne bacteria. *Eletronic Journal of Environmental, Agricultural and Food Chemistry.*8(**3**):195 – 200.
2. Ayatoa, J.F., Tchendem, M.H.K. and Nyasse, B. (1994). Novel bioactive diterpenoids from *Aframomum aulacocarpos. Journal of Natural Products* **57**: 917-923.
3. Bernard, D.L., Huffman, H.J., Meyerson, L.R. and Sidwell, R.W. (1993). Mode of Inhibition of respiratory syncytial virus by plant flavonoid. Chemotherapy **39**: 21-217.

4. Breslin, Andrew (2017). "The Chemical Composition of Green Plants". Sciencing, Leaf Group Ltd.
5. Caston, J.A. (2008), “Infections Bursal Disease virus (IBDV).” (HTTP: //WWW. horizon press. Com/Ma). Segmented Double Stranded RNA viruses: structure and Molecular Biology. Caster *Academic Press*. ISBN 978 – 1 – 90 4455 – 21 - 9
6. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, Pizzolatti MG, Silva FR (2008). "Flavonoids: Prospective Drug Candidates". Mini-Reviews in Medicinal Chemistry. **8** (13): 1429–1440. doi:10.2174/138955708786369564. PMID 18991758.
7. Cushnie TP, Lamb AJ (2011). "Recent advances in understanding the antibacterial properties of flavonoids". International Journal of Antimicrobial Agents. **38** (2): 99–107. doi:10.1016/j.ijantimicag.2011.02.014. PMID 21514796.
8. Dalziel, J. M., (1995). The useful plants of West Tropical Africa. *Crown Agents,* London. Pp. 364.
9. Eisikowitch, D.; Rotem, R. (1987). "Flower Orientation and Color Change in Quisqualis indica and Their Possible Role in Pollinator Partitioning". Botanical Gazette. **148** (2): 175. doi:10.1086/337645. JSTOR 2995472.
10. Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* **60** : 1-8. *Food Chemistry*. 57(2): 229 – 232
11. Foerster, Hartmut (22 May 2006). "MetaCyc Pathway: saponin biosynthesis I". Retrieved 23 February 2009
12. Galeotti, F; Barile, E; Curir, P; Dolci, M; Lanzotti, V (2008). "Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity". Phytochemistry Letters. **1**: 44–48. doi:10.1016/j.phytol.2007.10.001
13. Ghoshal, S., Krishna , B.N. and Lakshmi, V. (1996). Anti amoebic activity of Piperlongum fruits against Entamoeba histolytica *in vitro* and *in vivo. Journal of Ethnopharmacology.* **50**: 167-170.
14. Gill, L.S. (1992). Ethnomedical uses of plants in Nigeria. Uniben press, University of Benin, Benin – city, Edo State, Nigeria. ISBN 978 – 2027 – 20 – 0 Pp. 102 – 103.
15. Hasegawa, H. Matsumiya, S., Uchiyama, M., Kurokawa, T., Inovye, Y., Kasai, R., Ishibashi, S. and Yamasaki, K. (1994). Inhibitory effect of some triterpenoid saponins on glucose transport in tumor cells and its application *to invitro* cytotoxic and antiviral activities. *Planta Medica*. **6**: 240 - 243
16. Hostettmann, K.; A. Marston (1995). Saponins. Cambridge: Cambridge University Press. p. 3ff. ISBN 0-521-32970-1. OCLC 29670810.

17. Katie E. Ferrell; Thorington, Richard W. (2006). Squirrels: the animal answer guide. Baltimore: Johns Hopkins University Press. p. 91. ISBN 0-8018-8402-0
18. Kaul, T.N., Middle town, E.Jr. and Ogra, P.L. (1985). Antiviral effect of flavonoids on human viruses. Journal of Medical Virology. **15**: 71-79
19. Kittakoop P, Mahidol C, Ruchirawat S (2014). "Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation". Curr Top Med Chem. **14** (2): 239–252. doi:10.2174/1568026613666131216105049. PMID 24359196.
20. Krishnaiah, D., Devi, T., Bonno, A. and Sarbartly, R. (2009) Studies on phytochemical constituents of six Malaysian plants. *Journal of medicinal Plant Research*. **3**: 67 – 72.
21. Lemay, Marie-France. "Iron Gall Ink". Traveling Scriptorium: A Teaching Kit. Yale University. Retrieved 18 January 2017.
22. Marion Kite; Roy Thomson (2006). Conservation of leather and related materials. Butterworth-Heinemann. p. 23. ISBN 978-0-7506-4881-3.
23. McMahon, J.B., Currens, M.J., Gulakowaki. R.J., Buckheit. R.W.J., Lackman- Smith, C., Hallock, Y.F. and Boyd, M.r. (1995). Mochellamine B, A Novel plant alkaloid, inhibits human immunodeficient virus – induced cell killing by at least two distinct mechanisms. *Antimicrobial Agents Chemotherapy*. **39**:484-488.
24. McMillan, J.K., Cochran, M.D. Junker, D.E., Redd, D.N., and Valencia, D.M., (1994). The safe and effective use of fowl pox virus as a vector for poultry vaccines. *Development Biological Standard.* **82**: 137 – 165.
25. Mendooza, L., Wikkens, M. and Urzua, A. (1997). Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean Pseudognaphalium(Asteraceae). *Journal of Ethnopharmacology.* **58** : 85-88.
26. Mikail, H.G. (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. *bulbs* in experimental rabbits. *Journal of Medicinal Plant Research* **4**: 322 – 326.
27. Molyneux, RJ; Lee, ST; Gardner, DR; Panter, KE; James, LF (2007). "Phytochemicals: the good, the bad and the ugly?". Phytochemistry. **68** (22–24): 2973–85. doi:10.1016/j.phytochem.2007.09.004. PMID 17950388
28. National Research Council (2008). "Ebony". Lost Crops of Africa: Volume III: Fruits. Lost Crops of Africa. **3**. National Academies Press. ISBN 978-0-309-10596-5. Retrieved 2008-07-25.
29. Nonaka, G.I., Nishioka, I., Nishizawa, M., Yamagishi, T., Kashiwada, Y., Dutschman, G.E., Bodner, A.j., Kilkushie, RE., Chang, Y.C. and Lee, K.H.(1990). Anti-AIDS

agents, 2. Inhibitory effects of tannins on HIV reverse transcriptase and HIV replication in H9 Lymphocyte cells. *Journal National Products*. **53**:587-595.

30. Okwu, D.E.(2004). Phytochemicals and vitamine content of indigenous spices of South Eastern Nigeria. *Journal of Sustaining Agricultural Environment*. **6**: 30 – 34
31. Omulokoli, E., Khan, B. and Chhabra, S.C. (1997). Antiplasmodial activity of four Kenyan Medicinal Plants. *Journal of Ethnopharmacology*. **56**: 133-137.
32. Pengsuparp, T., Cai, L., Constant , H., Fong, H.H., Lin, L.Z., Kingborn, A.D., Pezzuto, J.M., Cordell, G.A., Ingoifsdottir, K. and Wagner, H. (1995). Mechanistic evaluation of new plant-derived compounds that inhibit. HIV-I reverse transcriptase. *Journal of Natural Products*. **58**: 1024-1031.
33. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A (2013). "Multitarget drugs of plants origin acting on Alzheimer's disease". Curr Med Chem. **20** (13): 1686–93. doi:10.2174/0929867311320130008. PMID 23410167
34. Scalbert, A (1991) Antimicrobial properties of tannins. *Phytochemistry* **30**: 38753883.
35. Serafini, M., Ghiselli, A. and Ferro-Luzzi, a (1994) Red wine, tea and antioxidants. *Lance*t. 344:626.
36. Shaanika, Helvy (2012). "Ombike – a potent traditional brew". New Era. Archived from the original on 28 October 2012
37. Uruquiaga,I.and Leighton, F.(2000). Plant polyptenol antioxidants and oxidative stress. *Biological Research* **33**: 159 – 165.
38. Venter, F. and Venter , J.A. (1996) .Making the most of indigenous trees. Briza publications, Pretoria. **1**(1): 24 – 30.
39. Xu, Ran; Fazio, Gia C.; Matsuda, Seiichi P.T. (February 2004). "On the origins of triterpenoid skeletal diversity". Phytochemistry. **65** (3): 261–291. doi:10.1016/j.phytochem.2003.11.014.
40. Yamamoto Y, Gaynor RB (2001). "Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer". Journal of Clinical Investigation. **107** (2): 135–42. doi:10.1172/JCI11914. PMC 199180 . PMID 11160126.