Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Open Access Journal available at: https://cajost.com.ng/index.php/files and https://www.ajol.info/index.php/cajost/index

DOI: https://dx.doi.org/10.4314/cajost.v5i2.18

Article Info

Received: 1st February 2023

Revised: 2nd June 2023

Accepted: 4th June 2023

In vitro activity of the ethanolic extracts of *Azadirachta indica* on the management of some fungal diseases affecting carrots in Sokoto state, Nigeria

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This study investigated the effectiveness of the seeds of *Azadrachta indica* plant extract as botanical antimicrobials on some fungi isolates associated with carrot plant diseases in Sokoto State of Nigeria. The fungi were isolated from diseased carrot plants from selected farms (Moreh, Badageni and Ruggar Liman farms) in the area. *Azadrachta indica* plant was extracted using ethanol. The common fungi isolated were *Alternaria alternata*, *Fusarium oxysporum, Mucor hiemalis* and *Penicillium notatum.* The seeds extract of the plant produced a zone of inhibition of 16.33 mm ± 2.52 mm against *M. hiemalis*. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the plant extract on the fungi isolated were then determined. The MIC of the extract tested against the organisms inhibited the growth of the fungi at a minimum inhibitory concentration of 6.3 mg/ml for all the organisms. The highest Minimum Fungicidal Concentration was 12.5 mg/ml on *A. alternata* while the least MFC was on *A. alternata* at 6.3mg/ml. The MIC and MFC values were observed to vary with the type of organism tested. The findings revealed that *Azadrachta indica* could be effective in the treatment of fungi associated with carrot plant. The study recommends that the in-vivo study of the plants effectiveness in treating carrot diseases be undertaken.

Keywords: *A. alternata carrot, ethanol, fungal,* MIC*,* MFC.

Cite this: *CaJoST,* 2023, 2, 225-231

1. Introduction

The ever rising growth in population, climate change, insecurity and pandemics are contributing to food shortages globally. One other factor is plant diseases that have prevented plants from performing optimally. These plant pathogens cause diseases that tend to reduce the plant performance in terms of yield and quality (Oerke and Dehne, 2004; Oerke, 2005). It has been observed that these losses are more in tropical countries where the environmental conditions favour the thriving of the pathogens (Oerke, 2005). Various pest control strategies have been adopted to reduce this negative trend mostly using chemical pesticides. This method has effect on the plants, land and the consumers (Rabi'atu *et. al.,* 2019). A more environmentally friendly and less harmful alternative is the use of natural products derived from plants (Aisha *et al,* 2013).

Carrot plant (*Daucus carota* L.) which is a member of the *Apiaceae* family is a very popular root vegetable grown worldwide. Carrot plants production is often affected by numerous diseases that significantly results in yield losses. Apart from the yield, such diseases also affect the crops nutritional value, shelf life, resistance to diseases and aesthetic appearance (Liliane and Charles (2020). Depending on environmental conditions, cultivation technology and other factors, yield losses can be as high as 40–60% (Oerke and Dehne, 2004; Oerke 2005). The economic and health impact of all these can be enormous. Here in Sokoto, Nigeria, carrot is widely cultivated during the dry season mainly in areas where there is abundant water. Various literatures have shown that some plant extracts can be effective in curbing plant pathogens (Aisha *et. al.*2019). Drost, (2010) Shrivastava and Singh*, (*2011); Aisha *et. al.,* (2019); and Hauwa and Aisha (2023) have all reported that some plants extracts could effectively suppress *Aspergillus niger*, *Aspergillus ochraceus*, and *Fusarium proliferatum.* Sanusi (2006) also reported a good antifungal activity of some plants against *Salmonella enteritidi.* To date, a wide range of medically active agents have been isolated from different parts of plants as extracts and used as sources of many potent

and powerful drugs (Shrivastava and Singh, 2011; Vieira *et al*., 2014 and Aisha *et. al.,* 2019). Some drugs that have been developed from plants and are still in use today include the analgesic drug aspirin, from *Filipendular ulmaria; the* anti-malarial agent, quinine from *Cinchona* spp; antineoplastic alkaloid, vincristine from *Catharantus roseus* and *digitalis* leaf as remedy for congestive heart failure (Sanusi, 2006). It is therefore important to scout for the diseases that infect plants and to find a healthy way to curtail their effect on food production.

2. Materials and Methods

2.1 Isolation and identification of fungi associated with carrot

Samples of infected carrots were randomly collected from Moreh, Badageni and Ruggar Liman farms in Sokoto state for fungal isolation and identification. This fungal isolation and identification was done at Usmanu Danfodiyo University, Sokoto following the procedure described in Rabi'atu *et. al*. (2023) and Gupta *et.al.* (2008). Sterilization of materials, inoculation room/chamber and media preparation were done as described by Cheesebrought, (2009). The fungi were then isolated based on the procedure described by Snowdon, (1990); Baiyewu *et al.* (2007); Chukwuka *et al.* (2010) and Onuorah *et al.* (2015).

2.2 Collection of Plant Samples

Fresh samples of the seeds of *Azadrachta indica* was obtained from Technical College Runjin Sambo, Sokoto State. The samples were packed separately in clean sterilized polythene bags and brought to the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, for identification and authentication. The Voucher numbers is UDUH/ANS/06.

2.3 Plant processing

The collected plant sample was separately processed into fine powder and stored in a polythene bag for further analyses. The processing involved separating the plant into parts before washing with clean tap water. The washed plant parts were then cut into small pieces and air dried in the laboratory (Aisha *et al*., 2013). The dried sample were then crushed using mortar and pestle, sieved through 0.28 µ mesh sieve before storing the fine powder in a polythene bag.

2.4 Qualitative analyses of the phytochemicals

Phytochemical analysis was used to determine the bioactive ingredients present in the extracts using standard qualitative methods. This was done to determine the presence of alkaloids, saponins, cardiac glycosides, flavonoids, steroids, tannins and anthraquinones as described by Harborne (1993); Soforowa (1993) and El-Olemyl *et al*. (1994).

2.5 Extraction of plants materials

Portion of the plant (100g) was weighed and soaked in 1liter of ethanol in a conical flask and left for 24 hrs. The content of the conical flask was filtered with Watman no. 1 filter paper and the filtrate was evaporated to dryness using hot oven at a temperature of 45^{o}C for 24hrs. The extracts were collected and used for antifungal tests.

2.6 Determination of percentage yield

After extraction, the yield of each extract was calculated by weighing the crude extract. The percentage yield was evaluated using the equation:

$$\% \text{ yield} = \frac{\text{Crude extract (g) X 100}}{\text{Total quantity (g)}}$$

2.7 Preparation of plant extracts concentration

Stock solution was prepared by dissolving 0.1g, 0.4g, 0.7g and 1g of the solid plant extracts in 5mls of a sterilized distilled water, making a stock of 20 mg/ml, 80 mg/ml, 140 mg/ml and 200 mg/ml respectively. The concentrations were used to test for the antifungal effect of the seeds extracts of *A. indica* of the stock of the extract were drawn using micropipettes and each suspended in a 6 mm hole.

2.8 Determination of *in vitro* activity of the plant extracts using agar well diffusion methods

The activity of the seed extract on the pathogenic fungal isolates was determined using Agar Well Method. The strains were maintained and tested on Sabouraud Dextrose Agar (SDA). Active cultures were generated by inoculating a loop full of culture in separate 10 ml SDA and incubating on a shaker at 20^{o}C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 mins, washed with normal saline, spun at 4000 rpm for 5 min. and diluted in normal saline to obtain 5x10cfu/ml.

2.9 Determination of antifungal activity of plants extracts using agar well diffusion method.

The crude extract of the plant sample was subjected to antifungal assay using the Agar Well Diffusion method of (McMurray *et al.,* 2011). 20 ml of Sabouraud Dextrose Agar was dispensed into sterile universal bottles and then inoculated with 0.2 ml of cultures mixed gently and poured into sterile Petri dishes. After setting a number 3-

cup borer (6mm) diameter was properly sterilized by flaming and use to make three uniform cups/well which were filled with 50ul of the extracts concentration of 20 mg/ml 80mg/ml, 140 mg/ml and 200 mg/ and allow diffusing for 45 minutes. The solvent used for reconstituting the extracts was similarly analyzed. The plates were incubated at 20^{o}C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates. The experiment was terminated when the control of each test filled up the Petri dishes (Mukherjee *et al.,* 2005).

2.10 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the micro tube method (Cheesbrough, 1991). Nutrient broth was prepared and sterilized in an autoclave at 121^{o}C for 15 minutes. Twelve test tubes were prepared. 1 cm^3 of the broth was dispensed into tube 2-12. A concentrated extract solution in sterilized water (3000 cm^3) was prepared. 1 cm^3 of the solution was dispensed in tube 1 and tube 2, from tube 2 serial dilution was carried out by transferring 1cm^3 up to tube 10 serially and from tube 10, 1cm^3 was taken and the content was discarded. Twenty-four hours (24hours) culture of each test organism was prepared in a sterile nutrient broth. 1:100 dilutions were transferred into each dilution tube, from the dilutions 1cm^3 was transferred into each tube from tube 1-12 with the exception of tube 2 to which (1 cm^3) of the sterile nutrient broth was added to make the final volume of each tube to be 2 cm^3. Tube 1 contained 1cm^3 of the extract solution and 1cm^3 of the inoculum; tube 2 contained 2 cm^3 of the nutrient broth only while tube 12 served as control for viability of the culture. The MIC was taken as the lowest concentration of the test compound that inhibits fungal growth after 24 hours' incubation at 27^{o}C.

2.11 Determination of Minimum Fungicidal Concentration (MFC)

The Minimum Fungicidal Concentration was determined by culturing the content of the tube that showed no visible growth in the MIC. A loop full of the mixture contained in the tubes were sub cultured on fresh prepared Sabouraud dextrose agar plate and incubated at ambient temperature for 7 hours (Duke and Martinez, 1994). The minimum fungicidal concentration was recorded as the concentration of extracts that did not permit any visible colony growth on the agar medium after the period of re-incubation (Wokoma *et al.*, 2008).

3. Results and Discussion

3.1 Results

The main fungi isolated were *Alternaria alternata*, *Fusarium oxysporum, Mucor hiemalis* and *Penicillium notatum* as the most common fungal agent of carrot disease in Sokoto state. The percentage yield of 6.76 was recorded. The phytochemical compound present in the seed of *A. indica* include Alkaloid, Glycoside and Flavonoids. Higher activity was recorded at higher concentration of 200 mg/ml against *Mucor hiemalis* and the least activity was found at the least concentration of 20 mg/ml against *Alternaria altanata*

Table 1 shows the Macroscopic and Microscopic features of the fungal isolates from diseased carrots. Four different colonies were identified based on the cultural and morphological features of the suspected isolates i.e *Alternaria alternata*, *Fusarium oxysporum, Mucor hiemalis* and *Penicillium notatum* as

The results obtained from the extraction is presented in table 2, the percentage yield of the crude extract of the seed of *A. indica* was 6.76.
The qualitative phytochemical analyses were presented in Table 3. The compounds present were alkaloids, saponins glycosides, glycosides, flavonoids, steroids and volatiles oil. Tannins and anthraquinones were not detected.
Table 4 shows the result of antifungal activity of the ethanolic extract of *A. indica* where the higher activity was recorded at highest concentration of 200 mg/ml.
Minimum inhibitory concentration and the Minimum fungicidal concentration were presented in table 5 and 6 respectively. MIC of 6.3 was recorded against all the organisms and the least MFC was also 4.3 against *Mucor hiemalis*

Table 1: Identification of fungal Isolates from the diseased carrots

S.No	Observed Isolates	Macroscopy	Microscopy
1.	*Alternaria alternata*	*Alternaria* spp grows as long chains with dark brown conidiophores.	Pale or dark brown conidiophores that may be straight or flexuous in appearance. Brownish conidia with short beak or no beak at all.
2.	*Fusarium oxysporum*	Aerial mycelium sparse or floccose, becoming felly, whitish or peach, usually with a purple tinge, more intense near the medium surface. Reverse in shades.	Micro-conidia 0 (-2) septate, borne on lateral, simple (often reduced) phialides.
3.	*Mucor hiemalis*	Colonies creamish yellow in daylight, more yellowish in darkness.	Sporangiophores simple at first, later slightly sympodially branched, often with yellowish bearing dark brown up to (85) m in diameter with deliquescent walls.
4.	*Penicillium notatum*	Texture velvety to powdery; green, blue, gray-green, white. The plate reverse is usually pale to yellowish.	Septate hyphae with branched or un branched conidiophores that have secondary branches known as metulae, on the metulae, arranged in whorls, are flask-shaped sterigmata that bear un branched chains of round conidia.

Table 2: Yield of crude extract of the seeds of *A. indica* using ethanol solvent

Plants extracts	Fraction	Weights (g)	Yield (%)
A .indica	Ethanol	6.8	6.76

Table 3: Qualitative phytochemical analysis of the seeds extracts of *A. indica,* in various solvents

Phytochemicals	Ethanol Fraction
Alkalonids	++
Tannins	ND
Saponin glycosides	+
Glycosides	+
Flavonoids	+
Steroids	+
Anthraquinones	ND
Volatile oils	+++

Key: + = present,
ND = Not Detected

Table 4: Antifungal activity of the ethanolic seeds extract of *A. indica*

Plant Extracts	Extracts Conc. (mg/ml)	Zone of Inhibition (mm)				
		A. niger	***A. alternata***	***F. oxysporum***	***M. hiemalis***	***P. notatum***
A. indica	20	-	4.33±0.5[b]	-	-	-
	80	-	6.0±0.0[b]	-	10.33±0.58[c]	9.0±1.73[c]
	140	-	6.33±0.5[b]	-	13.33±2.8[b]	12.0±2.0[b]
	200	-	7.56±1.3[b]	-	16.33±2.5[a]	12.33±2.9[b]
Ketoconazole	200	16.67±2[a]	18.67+1[a]	17.0±1.0[a]	15.0±0.0[a]	16.67±0.6[a]
Water	-	-	-	-	-	-
SE		0.67	0.55	1.71	2.98	3.26
P-Value		NA	0.00	0.014	0.02	0l.037
Significance		NA	*	*	*	*

Means followed by the same letter along the columns are not significantly different
* = significant at 5%,
Values=Mean ± SD,
SD= Standard deviation
S.E= Standard Error
NA= Not applicable
- = No activity

Table 5: Minimum Inhibitory Concentration (MIC) of ethanolic seed extracts of *A. Indica* against the fungal isolates.

Plant extracts	Organisms	Concentrations (mg/ml)								
		200	**100**	**50**	**25**	**12.5**	**6.3**	**3.1**	**1.7**	**MIC**
EAI	A. A	-	-	-	-	-	+	+	+	6.3
	M. H	-	-	-	-	-	+	+	+	6.3
	P. N	-	-	-	-	-	+	+	+	6.3

EAI=ethanol extract of *Azadirachta indica*; A. A= *Alternaria alternata*; M. H = *Muco rhiemalis*; P.N= *Penicilliu mnotatum*;- = no growth of test organism; + = growth of test organism.

Table 6: Minimum fungicidal concentration (MFC) of ethanolic seed extracts of *A. Indica* against the fungal isolates.

Plant extracts	Organisms	Concentrations (mg/ml)								
		200	**100**	**50**	**25**	**12.5**	**6.3**	**3.1**	**1.7**	**MFC**
EAI	A.A	-	-	-	-	+	+	+	+	12.5
	M.H	-	-	-	-	+	+	+	+	6.3
	P.N	-	-	-	-	+	+	+	+	12.5

EAI=ethanol extract of *Azadirachta indica*; A. A= *Alternaria alternata*; F.O = *Fusarium oxysporum;* M.H = *Mucor hiemalis*; P.N= *Penicillium notatum*;- = no growth of test organism; + = growth of test organism

3.2 Discussion

Isolation and identification of fungal pathogens associated with carrot in this research revealed four fungal species of *Aspergillus, Fusarium, Penicilliu Mucor* and *Alternaria.* This is in conformity with the findings of Hong and Pam (2001), that, the most common diseases on carrots produced in Tsamania were crown rot and cavity spot which are caused by *Fusarium* spp.

The ethanol extract of the plant gave a yield of 6.67 percent which compares well with the findings of Shina (2014) and Aisha, *et al*. (2019). Often non polar solvents yield more of lipophilic compounds whereas alcoholic and polar solvents yield both polar and moderately polar components (Yrjonen, 2004).

Phytochemical test was done the fraction and the results obtained are presented in Table 2. The result showed the presence of Alkaloids, Tannins, Saponins Glycosides, Flavonoids and

Steroids. The phytochemicals detected in the extract of *A. indica* is consistent with those reported by Shina (2014) which is an indication that *Azadrachta indica* could be effective in the treatment of fungi. This has also been supported by Odeja *et al.* (2015), who showed that ethanol is effective for extraction of phytochemicals and that ethanol extracts show high antimicrobial activity against organisms.

The seed extract of *A. indica* was tested for antifungal activity against *A. niger, A. alternata, F oxysporum, M. hiemalis* and *P. notatum.* The results of the tests are presented in Table 3. The results showed activity against *A. alternata, M. Hiemalis* sand *P. notatum* at concentration of 20, 80, 140 and 200 mg/ml. There was no activity at the lower concentration (20 mg/ml) in the *P. notatum* and *M. hiemalis.*

The ethanolic extract tested against the organisms inhibited the growth of the fungi at the minimum inhibitory concentration of 6.3 mg/ml (Table4). The Minimum Fungicidal Concentration (MFC) of the extracts was also determined against the fungi species (Table 5). The results showed that *Alternaria alternate* and *Penicillium notatum* have MFC of 12.5 mg/ml while *Mucor hiemalis* has the least value of 6.3 mg/ml. These were significantly ($P < 0.05$) different from the activity shown by the control (Ketoconazole) for all the organisms studied. This is in agreement with the findings of Ogie *et al.* (2010).

4. Conclusion

The study investigated the in-vitro activity of the ethanolic extracts of *azadirachta indica* on the management of some fungal diseases that infect carrot in Sokoto State, Nigeria. The study identified *Alternaria alternata*, *Fusarium oxysporum, Mucor hiemalis* and *Penicillium notatum*as the main fungi agents that infect carrot plants in Sokoto state. It also found that the ethanol extract of *azadirachta indica* could inhibit the growth of the isolated pathogens. The study recommends that in-vivo study of the effect of the plant extract on carrot plant diseases be carried out to further support the in vitro study. Other extraction methods should also be explored.

Conflict of Interest

The author declares that there is no conflict of interest.

References

Aisha. U. Bashir L. Aleiro, Lawal G. Hassan, Kasimu Shehu and M. S. Rabia (2019). Phytochemical study and antifungal activity of stem extracts of phyllantus niruri (L). *Caliphate Journal of Science and Technology*. ISSN: 2705-313 pp 12-18 DOI: 10.4314/cajost.v4i2.1

Aisha, U. and Aleiro B. L, (2013). Assessment of potentials of castor (ricinus Cummunis) Seed oil in the production of anticeptic detergent. *International journal of Research and Advancement in Physical Science*, Volume 3, Number 1 pp 125

Baiyewu, R. A., Amusa, N. A., Ayoola, O. A. and Babalola O. O. (2007). Survey of the postharvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L.) in south western Nigeria. *African Journal of Agricultural Research*, **2**(4): 178-181, ISSN 1991-637X

Cheesbrough, M. (1991). *Medical Laboratory Manual for Tropical Countries*, 2nd edition, Cambridge. Pp. 508-511, ISSN: 0950743410.

Chessbrough, M. (2009). *Districts Laboratory Practice in Tropical Countries*. 2nd Edition, Cambridge University Press, Pp33-34

.Chukwuka, K. S., Okonkol, O. and Adekunle, A. A. (2010). Microbial Ecology of organisms causing pawpaw (*Carica papaya* L.) fruit decay in Oyo State, Nigeria. *American-Eurasian Journal of Toxicological Science*,**2**(1): 43-50 DOI https://doi.org/10.2141/jpsa.0160116.

Drost, D. (2010). Peppers in the garden. Utah State University Cooperative Extension. Compendium of Pepper Diseases. *American Phytopathological Society Press*. Available at :http://www.apsnet.org/apsstore/shopapspress/Pages/43003. DOI Ihttps://doi.org/10.21273/HORTSCI.45.10.1464

Duke, J. A. and Martinez, R. V. (1994). *Handbook of Ethano botanicals.* Boca Raton (Peru): CRC press.

El-olemy, M. M., Almuhtadi, F. J. and Afifi, A. A. (1994). *Experimental phytochemistry;* A laboratory manual. King Saud University press. Saudi Arabia, Pp: 350-359. ISSN: 81-89422-30-8

Gupta, C., Amar, P., Ramesh, C., Uniayal, R. C. and Kumari, A. (2008). Comparative analysis of the anti-microbial activity of clove and cinnamon oil and extract on some food borne microbes. *African Journal of Microbiology Research*, **2**(9)**:** 247-251 DOI: 10.5897/AJMR11.400

Harborne, J. B. (1993). *Phytochemical Methods*: A Guide to Modern Technique of Plant Analysis. London: Chapman and Hall. Pp: 33-185

Hong, P. and Pam C. (2001). Horticultural research and development cooperation project VG96015

Liliane, T. N. and Charles S. M (2020). Factors Affecting Yield of Crops; *Agronomy Climate change food security.* DOI: 10.5772/intechopen.9067

McMurray, J. (2011). *Organic chemistry with biological applications (2nd edition)* Belmont. C.A Books/cole Pp. 395I-3999.

Mukherjee, K. P., Saha, K., Saha, P. B., Pal, M. and Das, J. (2005). Antifungal activities of the leaf extract of *Cassia tora* Linn. (Fam. Leguminosae). *Phytotherapy Research*, **10**: 521-522, ISSN 0951-418X

Odeja, O. O., Obi, G., Ogwuche, C. E. and Elenke, E. E (2015). *Phytochemical screening and antioxidant of Senna occidentalis* Leaves*. International Journal of Herbal Medicine,* **2**(4)**:** *Pp 26-30.*

Oerke, E. C. (2005). Crop losses to pests. *Journal of Agricultural Science*, **144**: 31–43. DOI: https://doi.org/10.1017/S0021859605005708

Oerke, E. C. And Dehne, H. W. (2004). Safeguarding Production Losses in major crops and the role of crop protection. *Crop Protection*, **23**: 275–285.Ogie, E. A., Eseigbe, D., Ilechie, M. N., Erhabor, I. and Ogbebor, E. (2010). Folial epidermal and phytochemical studies of the grasses (*Cymbopogon citrates*, *Axonopuscom pressus* and *Eragrostis tremula)* in Ekpoma, Edo State, Nigeria. *Science World Journal,* **5**(1):474-479. DOI: 10.4314/swj.v5i1.61481

Onuorah, S., Obika, I. and Okafor, U. (2015). Filamentous fungi associated the spoilage of post-harvest sweet orange fruits (*Citrus sinensis*) sold in Awka Major Markets, Nigeria. *Bioengineering and Bioscienc*e, **3**(3): 44-49. DOI: **10.13189/bb**.

Sanusi, H. W. (2006). The Antimicrobial activities, phytochemical analysis, toxicological and histopathological studies of some nine selected medicinal plants. A PhD Thesis submitted to the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto.

Shina, I. S. (2014). Phytochemical Analysis and Antimicrobial Activity of Some Medicinal Plants Used in Herbal Treatment in North Western Nigeria. A PhD Thesis submitted to the Department of Chemistry, Usmanu Danfodiyo University, Sokoto.

Snowdon, A. L. (1990). A colour atlas of post-harvest diseases and disorders of fruits and vegetables. *General introduction and fruits*. Wolfe Scientific Ltd, London, UK. English language,**1**: pp. 302.

Soforowa, A. (1993). *Medicinal plants and traditional medicine in Africa*. New York: Chichester John Wiley and Sons.

Shrivastava, D. and Singh, P. (2011). Antifungal potential of two common weeds against plant pathogenic fungi, *Alternaria* species. *Asian Journal of Experimental Biological Sci*ences, **2**(3): 525-528. https://doi.org/10.20546/ijcmas.2017.606.313

Vieira, D. R., Amaral, F. M., Maciel, M. C. Nascimento, F. R., Libério, S. A. and Rodrigues, V. P. (2014). Plant species used in dental diseases: ethnopharmacology aspects and antimicrobial activity evaluation*. Journal of Ethnopharmacology*, **155**(3): 1441-1449. DOI: 10.1016/j.jep.2014.07.021

Wokoma, E. C., Essein, I. E., and Agwa, O. K. (2008). The *in vitro* antifungal activity of garlic *(Allium satium*) and onion (*Allium cepa*) extracts against dermatophytes and yeast*. The Nigerian Journal of Microbiology*.**21**:21489

Yrjonen, T. (2004). Extraction and plant chromatographic Separation Techniques in the Analysis of Natural products. PhD Thesis Faculty of Pharmacy, University of Helsnk, Cosmoprnt, Helsnki, Finland, **(4)**: Pp.25-26.

