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ANTIFUNGAL Activity of Fruit Peels (exocarp) and Pulp (mesocarp) of African Bush Mango (*irvingia wombolu).*

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Abstract:- ***Irvingia wombolu*** **fruit is an economically important but underutilized fruit in Nigeria. Only the seed kernel of the fruit is of economic value as food and income source to the people, while the fruit peel and pulp are wasted. This study determined the yield weight of the ethanol and aqueous extracts of dried fruit peel and pulp of *I. wombolu* and also evaluated their antifungal activities against *Fusarium oxysporun f.sp. lycopersicum* (a wilt causing pathogen in tomato) *In-vitro* at three levels (1%, 3%, and 5%) of concentrations. The control experiments were sterile water and a synthetic fungicide. Each treatment and control was replicated thrice in a Complete Randomized Design. Data were taken of the radial mycelia growth of the pathogen at 24 hours interval for 96 hours. The results showed that the aqueous yield weights of the fruit peel and pulp extracts were higher than that of the ethanol extracts. All the extracts significantly (P<0.05) reduce the radial mycelia growth of the pathogen at varied levels compared with the negative control (sterile water) especially as from 48 hours of incubation. Antifungal activities of ethanol extract of the fruit pulp at all concentrations were comparable with the synthetic fungicide used. Aqueous extracts of the fruit peel and pulp were as effective against the pathogen as the fungicide only at 5% concentration. The antifungal property of the fruit peel and pulp of *Irvingia wombolu* should be exploited as a possible natural fungicide which is ecologically friendly and safe for use.**

Keywords:- *Antifungal activity; extract yield weight; Irvingia wombolu; peel and pulp extracts; radial mycelial growth.*

I. INTRODUCTION

Irvingia species are economically important but underutilized tree in the humid tropical rain forest zones of West and Central Africa (Antagana *et al*., 2001). The tree bears mango-like fruit called bush mango. There are two species of *Irvingia –I. gabonensis* which bears sweet edible fruit while *I. wombulu* bears bitter fruits (Mbaeyi-Nwaoha *et al*., 2017).. It is almost impossible to differentiate the trees of the two species until when they bear fruits (Mbaeyi-Nwaoha *et al*., 2017). The fruit has ellipsoidal shape, and it is green in colour when unripe, but turns yellow when ripe. The fruit has thin skin, juicy pulp and seed stone. The seed stone bears the seed kernel which is of great value when processed into powdery form used in preparation of slimy soup. In Nigeria, the soup is commonly called "Ogbonno" by the Igbos, "Aapon" by the Yorubas, and "Goronor" by the Hausas (Etta *et al*., 2014). Fruit pulp of *Irvingia gabonensis* is normally consumed raw and fresh because of the sweet taste it possess while fruit of *I. wombulu* is usually allowed to rot before the extraction of the seed kernel need for soup or for income generation. The fruit pulp of Irvingia wombulu is wasted because of the bitter taste of turpentine it contains (Etukudo, 2000).

Previous research work reported the presence of various phytochemicals in different plant parts like root, stem bark, leaves, and fruit of *Irvingia* species (Fadare and Ajaiyeoba, 2008; Oduro *et al*., 2015; Etebu, 2012; Etebu *et al*., 2014; Tungbulu *et a*l., 2016) .Some of the phytochemicals mentioned include alkaloids , flavonoids, saponnins, tannins and glycosides in varied quantities. These group of phytochemicals play very important role in human medicine and therapeutic (Banso and Adeyemo, 2007; Raji *et al*., 2001). Alkaloids was reported by Ashihara *et al*., (2008) and Agrios, (2005) as an important phytochemicals occurring in plant primarily to defend the plant against herbivore, pathogens like fungi, bacteria, viruses and other competing plants.

Both Irvingia gabonensis and I. wombulu contains the same type of phytochemicals, but the later has relatively higher quantities of alkaloids, saponins and tannins than I. gabonensis (Etebu, 2012; Etebu,2013; Tungbulu *et al*., 2016). Although the availability of any phytochemical in plant is influenced by genotype, maturity , environment and post-harvest conditions (Manach *et al*., 2004).The availability of the phytochemicals in *Irvingia* species has been exploited by few researchers in evaluating the antimicrobial activity of extracts from various part of the plant against some human and plant pathogens (Fadare and Ajaiyeoba, 2008; Etebu and Benjamin, 2014; Etebu and Tungbulu, 2015; Tungbulu *et al*, 2016; Nworie *et al*., 2016).These earlier works made use of *I. gabonensis*. The fruits of *Irvingia wombulu*- the bush mango with bitter taste, are usually left in a heap to allow the pulp to rot for easy

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extraction of the seed kernel which is the most valued part of the fruit. The pulp which makes up about three quarter of the total weight of the fruit is wasted.

Therefore this study aimed to determine the extract yield of dry fruit skin and pulp of *Irvingia wombolu* using water and ethanol as extracting solvents; and also evaluate their antifungal activities against *Fusarium oxysporum f. sp. lycopersicum* – a plant pathogen causing fungal wilt in tomato.

Figure 1 Fruits of *Irvingia wombolu*

II. MATERIALS AND METHOD

- *Plant material collection:*

Matured *Irvinga wombolu* fruits were obtained from the *Irvingia* orchard of Fruit Research Programme, National Horticultural Research Institute, Ibadan, Nigeria. They were washed and air dried. Exocarp (skin) and mesocarp of the fruits were removed separately using knife; and they were dried in a food dehydrator (Exacalibur, USA) at 52^0 C. The samples were then blended/ ground into powder separately and stored in glass bottles for use.

- *Pathogen collection:*

Fusarium oxysporum f.sp. lycopersici was obtained from the Pathology laboratory of the National Horticultural Research Institute (NIHORT), Ibadan. It was cultured on petri dishes for seven days before use in the in- vitro experiment.

- *Extracts preparation:*

Two extraction solvents used were water and ethanol. Into two sets of three conical flasks, 1g, 3g, and 5g of the powder (skin) and powdered pulp samples were poured, after which 99ml, 97ml, and 95ml of water was poured into each set of the flask respectively to have 1%, 3% and 5% concentrations according to Do, Q.D., et al, (2007)

- *Antifungal activity of I. wombolu extracts on radial mycelial growth of F. oxysporum In-vitro.*

Aqueous and ethanol extracts of the dried and blended skin (exocarp) and pulp (mesocarp) of *Irvingia wombulu* were evaluated at different concentrations for their efficacy against the radial mycelial growth of *F. oxysporumIn- vitro* according to George I. Nduke and Yimin Zhao (2007).

- *Statistical analysis:*

The effect of ethanol and aqueous extracts of skin and pulp of *Irvingia wombulu* at different concentrations on the radial mycelial growth of *F. oxysporum* was analysed by a one way analysis of variance (ANOVA) and mean differences between concentration levels of the plant extracts were separated by Fisher's Least significant difference (LSD) at 5% significant probability level using SAS, GLM procedure (SAS Institute, 2002)

III. RESULTS AND DISCUSSION

Differences in yield of aqueous and ethanol extracts of fruit skin and pulp of *Irvingia wombolu* were observed (Table 1).The yield from aqueous extraction is higher than that of the ethanol extraction for both exocarp and mesocarp of *Irvingia wombulu.* Aqueous extraction of 1%, 3% and 5% of fruit skin of *I. wombulu* yielded 0.23, 0.68 and 1.14 grammes while ethanol extraction yielded 0.08, 0.26 and 0.42 gramme respectively. The difference in yield of aqueous to ethanol is more than double for the fruit skin extracts. Aqueous extraction of 1g, 3g, and 5g of fruit pulp yielded 0.38, 1.08, and 2.3 grammes while extraction pf the same quantities with ethanol yielded 0.03, 0.08 and 0.14 grammes respectively. Aqueous yields of fruit pulp extraction were more than twelve times higher in values than ethanol yields. The difference in extract yields observed in this study may be attributed to the difference in polarity of the extracting solvents used i.e. water and ethanol, which will allow different components of phytochemicals present in the samples to be removed (Dai and Mumper, 2010). These findings are in agreement with previous investigations of Do *et al*., (2014), Liu *et al.* (2007). Sultana *et al.* (2009) reported that extract yield is strongly dependent on the nature of the extracting solvent which will allow different plant compounds (phytochemicals) with diverse chemical characteristics and polarities to dissolve or not in it. Polar solvents are usually used to extract polyphenols from plant materials.

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Table 1:Yield after extraction

Fruit part	Initial weight (g)	Yield weight (g)		Percentage yield	
		Aqueous	Ethanol	Aqueous	Ethanol
Skin	1	0.23	0.08	23.00	8.00
	3	0.68	0.26	22.67	8.67
	5	1.14	0.42	22.80	8.40
Pulp	1	0.38	0.03	38.00	3.00
	3	1.08	0.08	36.00	2.67
	5	2.03	0.14	40.60	2.80

Table 2: Antifungal activity of aqueous and ethanol extracts of fruit skin(exocarp) and pulp (mesocarp) of *Irvingia wombolu* on radial mycelial growth of*Fusarium oxysporum f.sp. lycopersicum*

Fruit part	Solvent	Concentration (%)	Radial mycelial growth (mm)			
			24 hours	48 hours	72 hours	96 hours
Skin(exocarp)	Ethanol	1	9.00bc	14.00bc	20.33b	29.00b
		3	9.33abc	14.00bc	20.00bc	25.67c
		5	9.67ab	13.67c	18.00d	24.00de
Pulp(mesocarp)		1	8.67c	12.00d	15.67e	20.67g
		3	7.67d	9.00e	10.67f	11.00i
		5	4.33e	5.67f	8.00g	9.67i
Skin(exocarp)	Water(aqueous)	1	9.00bc	14.67b	20.67b	28.67b
		3	9.67ab	14.33bc	20.67b	23.00ef
		5	9.00bc	11.67d	15.00e	19.00h
Pulp(mesocarp)		1	10.00a	14.00bc	18.00d	24.67cd
		3	9.33abc	13.67c	18.67cd	22.50f
		5	10.00a	14.00bc	17.67d	19.67gh
Control(negative)		Sterile distilled water	8.67c	15.67a	22.67a	31.00a
Fungicide		2.3g/litre	7.67d	11.67d	15.50e	19.17h
$LSD_{(0.05)}$			0.77	0.97	1.56	1.44

Note: Mean followed by the same letter in a column are not significantly different at P< 0.05 according to DMRT

There was no significant difference in the radial mycelial growth of *Fusarium oxysporum* when treated with both aqueous and ethanol fruit peel (exocarp) extracts of *Irvingia wombulu* at 24 hours of incubation (Table 2), but a significant difference was observed in fruit pulp extracted with ethanol and water. Ethanoic extracted fruit pulp has a better reduction in radial growth of the mycelia of the pathogen at 3% (7.67mm) and 5% (4.33mm) concentrations. Three percent concentration is comparable to the fungicide (7.67mm), while 5% concentration controls the growth of the pathogen better than the synthetic fungicide used as check. Aqueous extract of the fruit pulp encouraged the growth of the pathogen; but this growth is not significantly different from that of aqueous and ethanoic fruit skin extracts.

At 48 hours of incubation, all extracts at all concentrations significantly reduced of the radial mycelial growth (RMG) of the pathogen at varied levels (Table 2). Aqueous extract of the fruit skin at 5% showed antifungal activity that can be compared with the fungicide used because the RMG of the two were the same (11.67mm).Ethanol extract of the fruit pulp at 1%, 3% and 5% concentrations showed lower significant difference in RMG of the pathogen (12.00mm , 9.00mm and 5.67mm) than its aqueous extracts (14.00, 13.67 and 14.00 mm) respectively. This signifies a better control of the pathogen by the ethanol extract of the fruit pulp. The RMG of the ethanol extract of the pulp at 1% (12.00mm) can be compared with that of synthetic fungicide (11.67mm), while at higher concentrations of 3% and 5% they reduced the RMG to 9.00mm and 5.67mm respectively which are much lesser than that of synthetic fungicide (11.67mm)..

At 72 hours, all the extracts at all concentrations reduce the RMG of the pathogen significantly compared to the RMG of the control (sterile water). At 1% and 3% concentrations of fruit skin, there were no significant difference in RMG for both ethanol extracts (20.33 and 20.00mm) and aqueous extracts (20.67,and 20.67); but at a higher concentration of 5%, the RMG of it ethanol and aqueous extracts were 18.00mm and 15.00mm. The latter is comparable with the fungicide control (15.50mm). At 3% and 5% concentration of the ethanol extract of fruit pulp, the RMG of the pathogen were 10.67mm and 8.00mm which were significantly lesser than the RMG of the fungicide control of 15.50mm. This means that the 3%and 5 % concentrations of ethanol extract of the fruit pulp have more antifungal activiyty aginst tha mycelial growth of the pathogen tested than that of the fungicide used.

Aqueous and ethanol extracts of the fruit skin and pulp were effective in reducing the RMG of *Fusarium oxysporum* at all concentrations used in this study. They were all significantly different from the negative control. Five percent concentration of aqueous extract of the skin (19.00mm) and pulp (19.67mm) could be compared with the fungicide (19.17mm) in effectiveness in reducing the growth of the pathogen. Ethanol extract of the fruit pulp of *I. wombulu* at 3 and 5%were also effective and reduced the RMG of the pathogen than the synthetic fungicide used.

Generally, the ethanol extract of the fruit pulp was found to be more effective against the RMG of the *Fusarium oxysporum* throughout the hours of this study, though the yield quantity of the water /aqueous extract was more in quantity (Table 1). This implies that the quantity of yield does not determine the antifungal activity of an extract but the type of phytochemicals extracted by the extracting solvent (Qasim *et al.,* 2016) and the effectiveness of the extracted phytochemicals against the tested organism.

Ethanol extract of the fruit pulp of *Irvingia wombolu* was the most effective in reducing the RMG of the tested plant fungal pathogen. Fadare and Ajaiyeoba (2008) and Etebu and Benjamin (2016) reported effectiveness of plant parts of *Irvigia gabonensis* extracts against some bacteria and fungi. The results of this work is comparable to results of previous work of Etebu and Benjamin (2016) who reported the potentials of extract of *Irvinga gabonensis* fruit pulp waste to manage some post-harvest spoilage fungi especially Aspergillus and Mucor species. This effectiveness could be attributed to the varied amount of phytochemicals present in different parts of *Irvingia* species (Fadare and Ajaiyeoba, 2008; Oduro *et al.,* 2015; Etebu, 2012; Etebu *et al*., 2014; Tungbulu *at al.*, 2016). From this work, the active phytochemical(s) (alkaloids) against the tested pathogen was /were greatly extracted by the ethanol. Qasim *et al.* (2016) reported the effectiveness of aqueous organic solvents like ethanol, methanol, acetone etc.in extracting phytochemicals and antioxidants from plant materials.

Aqueous extract of the fruit skin at high concentration of 5% was observed to be effective against the growth of the pathogen. Peels of fruits generally contains many phytochemicals which are antimicrobial in activity (Al-Zoreky, 2009).

Further work involving the combination of both the dry fruit peel and pulp together and the evaluation of their antifungal property in screen house and field experiments is necessary.

IV. CONCLUSION

Instead of allowing the fruit pulp of *Irvingia wombulu* to rot before the extraction of the seed kernel that is presently assumed as the only part of the fruit that is of great value as a source of food and income to the people, the antifungal property of the fruit pulp should be exploited and use as a possible natural fungicide which is ecologically friendly and safe for use.

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