EFFECT OF FICUS EXASPERATE LEAVES EXTRACT ON PALM WINE FERMENTATION

Ogbuanu, Cyril Chinedu and Amujiogu, Steve Nnaedozie

Enugu State University of Science and Technology, Department of Industrial Chemistry, P.M.B 01660, Enugu Nigeria

Introduction

Studies on the inhibition or regulation of the microbial activities and to extend the self-life of the palm wine have been on. The use of chemical additives and addition of plant material and extracts have greatly affected the organoleptic quality and quantity of the product (Iheonu, 2000; Nwokeke, 2001; Obire, 2005; Chime, Ogbuanu & Attah, 2007). Phytoconstutents of edible and medicinal plants have been reported to posses significant antimicrobial activities (Mishra, Kehri, Sharma, & Pandey, 2009; Mishra, Singh, Gupta, Chateervedi, Pandey, Tiwari & Mohapatra, 2013; Meda, Bangou, Bakasso, Millogo-Rasolodimby & Nacoulma, 2013; Cushine and Lamb, 2005).

The activities of Saccharomyces cerevisiac as the dominant micro-organisms responsible for the fermentation of palm wine (Marzia, Francesca, Manuela & Maurizio, 2009) among other yeast species (Amoa-Awua, Sampson & Tano-Debrak, 2006) has been studied when dosed with chemicals as preservatives (Okafor, 1974; Agu, Ogbuanu, Ezema, Onyia, Ude & Nwankwo, 2000) and plant materials or extracts (Chime, Ogbuanu & Attah, 2007; Ekop, Eyop & Elemike, 2012). The results showed that the rate of fermentation as volume of CO_2 evolved, the percentage of alcohol, and other parameters were either increased or decreased as a result of enhancing or inhibiting the micro-organism activity.

This study aimed at accessing the effect of varying concentration of n-hexane, ethyl acetate and methanol extracts of *Ficus exasperate* (Awariwa) leaves on the fermentation of palm saps.

MATERIALS AND METHODS, Sample Collection

Fresh leaves of *Ficus exasperate* were collected from surrounding of Enugu State University of Science and Technology, Agbani. Prof. J. C. Okafor, a taxonomist, Dept. of Applied Biology and Biotechnology, ESUT authenticated the plant. The leaves were shade dried and powdered. Palm sap sample (*Elaeisguineensis*) was collected by special commissioned tapper to avoid adulteration and dilution from Udi in Enugu State.

Chemicals

n-Hexane, ethyl acetate and methanol and all the chemicals used for phytochemical analysis were purchased from FinLab Nigerian Ltd. The chemical and solvent used were analytical grade.

Qualitative Phytochemical Analysis

The n-hexane, ethyl acetate and methanol extracts were subjected to various qualitative phytochemical tests to determine the active constituents present in the crude extracts using the procedures of Sofowora, Trease and Evans (Sofowora, 1993; Trease and Evans, 1978, 1989, 2002). The phytochemicals tested for were: saponin, glycosides, phytosterol/ triterpenoids, anthiracenes, tannins, flavonoids, resins, volatile oil and alkaloids compounds.

Fermentation Studies of Palm Sap

The experiments were set up as described earlier (Agu, Okendii, Ude, Onyia, Onwumelu & Ajiwe, 1999). Exactly 100mL of *E. guineensis* sap, were dosed with 0.0, 2.5, 50 and 100mg on the extracts of *Ficus exasperate* leaves. The rate of fermentation was determined at 32^0C (room temperature) by monitoring the volume of carbon dioxide evolved during fermentation and reading taken every 1hr.

Total Reducing Sugar of Palm wine

Fehling's solution method as described by Anon 1970 was used to determine total reducing sugar content of the sap and 24 hr fermented palm wine respectively. Twenty five milliliter of mixed Fehling's solution A and B (50/50 v/v) was titrated with 25mL of water while boiling with standard glucose solution (200mg/250mL) using methylene blue as indicator. The process was repeated with fresh palm sap and 24 hr fermented palm wine respectively.

Determination of Percentage Alcohol

Hundred milliliter of the fermented palm wine and those dosed with various concentrations of the extracts were distilled with 50mL of distilled water respectively. The volume of 95mL of the distillate was made up to 100mL with distilled water and the percent alcohol determine using Gay Lussac alcohol meter (Model No. 6181).

RESULTS AND DISCUSSIONS

Table 1 shows the preliminary phytochemical profile of the n-hexane, ethyl acetate and methanol leaves extracts of *F. experata*. The phytochemicals screening showed that the leaves extracts contain alkaloids, resins, tannnis, phytosterol/ tritterpenoids and saponin.

Table 1: Preliminary Phytochemical analysis of n-hexane, ethyl acetate and methanol extracts of *F. experata* leaves.

Phyto chemicals	n-Hexane	Ethylacetate	Methanol
Alkaloids	-	-	+
Resins	+	-	-
Tannins	-	-	+
Phytosterol/triterpenoids	+	+	-
Saponin	-	+	-
Volatile oil	+	-	-

Key: + = positive; _ = absent

Alkaloids and Tannins were present in methanol extract and absent in n-hexane and ethyl acetate extracts, while resins and volatile oil was showed to be sterol/triterpenoid was observed in n-hexane and ethyl acetate extracts but absent in methanol extract. Saponin was only observed in ethyl acetate. Phytochemicals detected in this plant, possess various biological activities which may help in inhibiting/suppressing microbial activity in palm wine fermentation.

Table 2: Volume of CO_2 evolved during fermentation of palm wine dosed with various concentrations of n-Hexane, ethyl acetate and methanol extracts of *Ficus exasperate* respectively.

Fermentation Period (s) ($X10^3$)	Control 0.0mg	n-Hexane extract 25mg	n-Hexane extract 50mg	n-Hexane extract 100mg	Ethyl acetate extract 25mg	Ethyl acetate extract 100mg	Methanol Extract 25mg	Methanol Extract 50mg	Methanol Extract 100mg
0	40 (0.00)	40 (0.00)	60 (0.00)	30 (0.00)	90 (0.00)	70 (0.00)	40 (0.00)	40 (0.00)	50 (0.00)
3.6	50 (0.04)	50 (0.04)	65 (0.02)	35 (0.02)	100 (0.04)	80 (0.04)	45 (0.02)	45 (0.02)	55 (0.02)
7.2	55 (0.06)	55 (0.06)	75 (0.06)	45 (0.06)	110 (0.08)	85 (0.06)	50 (0.04	50 (0.04)	60 (0.04)
10.8	60 (0.08)	60 (0.08)	80 (0.08)	50 (0.08)	115 (0.10)	90 (0.08)	55 (0.06)	55 (0.06)	65 (0.06)
14.4	65 (0.10)	65 (0.10)	85 (0.10)	55 (0.10)	120 (0.12)	95 (0.10)	60 (0.08)	60 (0.08)	70 (0.08)
18.0	70 (0.12)	70 (0.12)	90 (0.12)	55 (0.10)	130 (0.16)	100 (0.12)	65 (0.10)	65 (0.10)	75 (0.10)
21.6	75 (0.14)	75 (0.14)	95 (0.14)	60 (0.12)	135 (0.18)	105 (0.14)	70 (0.12)	70 (0.12)	80 (0.12)
25.2	80 (0.16)	80 (0.16)	100 (0.16)	65 (0.14)	140 (0.20)	110 (0.16)	75 (0.14)	75 (0.14)	83 (0.13)
28.8	85 (0.18)	85 (0.18)	100 (0.16)	70 (0.16)	145 (0.22)	115 (0.18)	80 (0.16)	77 (0.15)	85 (0.14)

Fig. 1: Fermentation profile of various concentration of n-hexane extract dosed to 100mL of palm wine sap against control

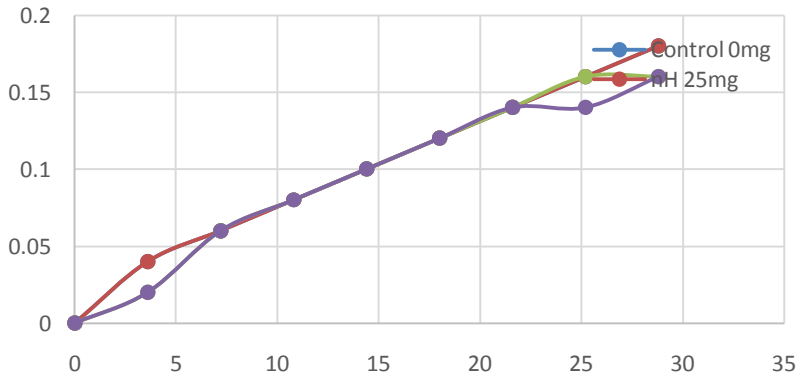
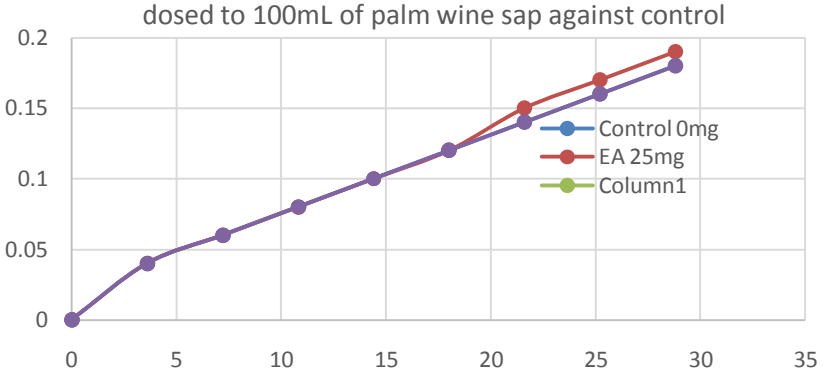
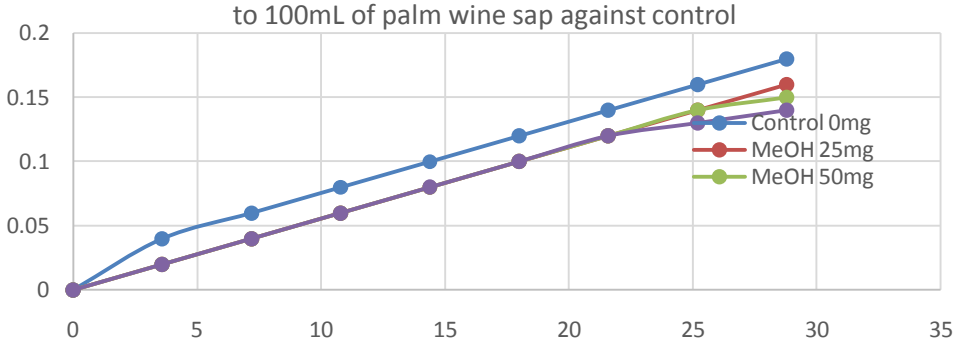


Fig. 2: Fermentation profile of various concentration of ethyl acetate extract dosed to 100mL of palm wine sap against control



Fermentation profile of various concentration of methanol extract dosed to 100mL of palm wine sap against control



The results obtained when different concentration of the n-hexane, ethyl acetate and methanol extracts (25mg, 50mg and 40mg) of *F. experata* leaves were dosed to the palm saps to inhibit bacterial activity during fermentation are shown in Fig. 1, 2 and 3.

There is no significant change in the volume of CO_2 produced for the control and all concentration of n-hexane and ethyl acetate extracts. (Table 2). This suggests that the n-hexane and ethyl acetate extracts neither inhibit nor enhance fermentation microbes in palm wine. However, the volume of CO_2 evolved in pure *Elaeis* saps (control) varied remarkably from those dosed saps with methanol extracts. The decrease in the volume of CO_2 evolved showed that methanol extract acts as inhibitor to the fermentation microbes.

The observed general decrease in the rate of fermentation of the saps with the addition of methanol extract of F. exasperate might be attributed to the decreased activities of microbes in the saps (fig. 3). It is likely that alkaloids and tannins constituents of the methanol extract of *F. experata* seem to be inhibiting the microbes and hence slowing down the fermentation process at the three concentrations studied. This is suggested that the inhibition will be optimal as lower concentration. However, with respect to the amount of alcohol produced, it was found that the control and n-hexane and ethyl acetate dosed experiments generated the same amount of alcohol more than the methanol dosed samples (Table 3). It is possible that methanol extract inhibits the rate of fermentation by making the microbes to convert the sugar slower into alcohol and other products and methanol extract inhibited microbial activities (Fig. 1, 2, 3).

The percentage of alcohol produced during the fermentation of the pure and various concentrations of methanol dosed saps show clearly that for every time between 0.00 – 1.0 x 10^{-6} seconds, the percentage of alcohol decreased more rapidly in the methanol dosed than the pure saps (Fig. 3). The rapid decrease in percent alcohol supports the observed decrease in the volume of CO_2 produced in the methanol dosed samples and might be a result of the deactivation of the microbes.

Table 3. Percent alcohol and rate constant for the fermentation of Elaeis saps dosed with 25, 50 and 100mg of *F. experata* leaves extracts

Extracts	% Alcohol	Rate constant (X10^{-6} $mol^{-1}Sec^{-1}$)	Total reducing sugar
Control (0mg)	6	6.667	8.19
n-Hexane (25g)	6	6.667	-
n-Hexane (50g)	6	6.667	-
n-Hexane (100g)	6	6.667	-
ethyl acetate (25g)	6	6.667	-
ethyl acetate (50g)	6	6.667	-
ethyl acetate (100g)	6	6.667	-
Methanol (25g)	4	5.556	-
Methanol (50g)	4	5.556	-
Methanol (100g)	4	5.556	-
Control after 24h	-	-	1.33

Conclusion

The present study has shown that methanol extract of *F. experata* leaves inhibits the rate of fermentation of *Elaeis* sap and decreases the amount of alcohol produced. It has also shown that the addition of n-hexane and ethyl acetate extracts does not influence appreciably its fermentation properties with that of the pure sap (control). This has shown that methanol extract of *F. experata* leaves extract suppress the microbial activities and fermentation of the pure sap and has opened fresh grounds for more study into the preservation of *Elaeis* sap.

References

Agu, R. C., Ogbuanu, C. C., Ezema, P. O., Onyia, A. I, Ude, C. M. & Nwankwo, Y. I. (2000). Effect of bacteria inhibition on the fermentation kinetics of Elaeisguineensis. *J. Food Sci and Technol 37*(6): 648-650.

Agu, R. C., Okendii, M. U., Ude, C. M., Onyia, A. I., Onwumelu, A. H., & Ajiwe, V.I.E. (1999). Fermentation kinetics studies of Nigerian palm wine *Eleaisquineenisi* and *Raphiahookeri* for preservation by bottling. *J. Food Sci. Technol 36*: 205-209.

Amoa-Awua, W. K., Sampson, E. & Tano-Debrak, K. (2006). Growth of yeast lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeisguineensis) in Ghana. *Journal of Applied Microbiology, 102* (2): 599-606.

Chime C. C., Ogbuanu C. C. and Attah J. C. (2007). The effect of Hausa kola-nut (kola nitida) on the fermentation of Elaeisguineensis and Raphiahookeri. *J. Sci. Engr. Tech. 14*(1): 7217-7225.

Cushine, T. P. T. & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5): 343-356.

Ekop, A. S., Eyop, N. D., & Elemike; E. E. (2012). Effect of lemon juice on the kinetics of fermentation of palm wine. *Asian Journal of Science and Technology, 4*(11): 17-19.

Iheonu R.T. E. (2000). Effect of local preservatives of plant origin on microbial and shelf life stability of palm wine. B.sc. Thesis submitted to Abia State University, Uturu, Nigeria.

Marzia, S., Francesca, C., Manuela, T. & Maurizio, C. (2009). Yeast diversity during tapping and fermentation of palm wine from Cameroon. *Food Microbiology 26*(4): 415-420.

Meda, N. T. R. Bangou, M. J.; Bakasso, S., Millogo- Rasolodimby J. & Nacoulma, O. G. (2013). Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandraandmaeruaangolensis* of Burkina Faso, *Journal of Applied Pharmaceutical Sciences, 3* (02): 036-042.

Mishra P. K.; Singh, R. K; Gupta, A; Chateervedi A; Pandey, R; Tiwari, S. P. & Mohapatra, T. M. (2013). Antibacterial activity of Andrographispaniculata (Burm. f.) Wall ex Nees leaves against clinical pathogens. *Journal of Pharmacy Research, 7*(5): 459-462.

Mishra, A. K., Mishra A, Kehri, H. K. Sharma, B. & Pandey, A. K. (2009). Inhibition activity of India spice plant *Cinnamomumzeylanicumertrats* against *Alternariasolani* and *Curvularia*lunata, the pathogenic dematiaceousmoulds. *Ann. Clin. Microbiol. Antimicrol*. 8, 10. 1186/1476-0711-8-9.

Nwokeke N. V. (2001). Palm wine preservation using traditional plants that have preservative bases. B.Sc. Thesis submitted to Imo State University Owerri, Nigeria.

Obire, O. (2005). Activity of zygomonas species in palm sap obtained from three areas in Edo State Nigeria. *Journal of Applied Sciences Environmental Management, 9*: 25-30

Okafor, N. (1975). Microbiology of Nigeria palm wine with particular reference to bacteria. *J. Appl Bact., 38* (2): 81-88.

Sofowara, A. (1993). Screening plants for bioactive agents: Medicinal plants and traditional medicine in Africa. 2nd Edn. Ibadan: Spectrum Books Ltd.

Trease, G. E. and Evans W. C. (1989). *Pharmacognosy. 13th edition*. London: ELBS/ Bailliere Tindal

Trease, G. E. and Evans W. C. (2002). *Pharmacognosy. 15th Edition.* London: WB Saunders, London.

Trease, G. E. and Evans, W. C. (1978). *A textbook of pharmacognosy, 11th edition*. London: Bailliere Tindal