TITLE PAGE

PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF HULLS AND NUTS OF Tetracarpidium conophorum (ukpa)

 \mathbf{BY}

UKPAI, EKENEM GRACE

PG/AMB/M.Sc./2006486012P

A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED

MICROBIOLOGY AND BREWING IN THE FACULTY OF BIOSCIENCES, AS

PART OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF

SCIENCE (MEDICAL MICROBIOLOGY) DEGREE OF NNAMDI AZIKIWE

UNIVERSITY, AWKA.

DECEMBER, 2010

CERTIFICATION

Ukpai, Ekenem Grace, a post graduate student in the Department of Applied Microbiology and Brewing and has satisfactorily completed the requirement of course and research work for the degree of Master of Science (M.Sc.) in Medical Microbiology.

The work embodied in her thesis is original and has not been submitted in part or full for any other diploma or degree in this or any other University.

Prof. (Dame) E.I. Chukwura

Supervisor

Dr Chris Anyamene

Head of Department

Applied Microbiology

and Brewing,

Nnamdi Azikiwe

University, Awka.

DEDICATION

This work is dedicated to the Almighty God, my sweet darling husband Mr Chancel Ukpai and children, Hadassah and Praise.

ACKNOWLEDGEMENT

I am deeply indebted to my love for life Mr Chancel N. Ukpai for his love, moral, physical and financial support throughout my course and project works. My wonderful angels: Hadassah and Praise, thanks for your love and understanding. I am also highly indebted to my parents, in-laws, for all their contributions throughout the course of my study.

I wish to express my special appreciation to my supervisor, Prof. (Dame) E.I. Chukwura for her ardent interest in this research work.

I am grateful to all academic and non-academic staff of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka especially the present Dean of Faculty of Biosciences, Prof. J.F.C. Odibo for all the assistance rendered towards the achievement of this dream.

I also thank Dr Stan Udiedi, Mr Anagonye, Miss Benne Anajekwu, and all the staff of Biochemistry, Industrial Chemistry laboratory for their selfless services and love shown me during this research work.

I extend my special thanks to my friends, Mrs Onyi Ezeh, Mrs Uju nee Obiefuna, Mrs Ngozi Ndeche, Mr Chukwudi Onwusi, Mrs Ekwealor, Mrs Anyoha. Mr Henry, all my course mates to mention but a few who made the academic period interesting.

Finally, I wish to thank my Computer Operator, for being available to typeset the manuscript.

ABSTRACT

Phytochemical and antimicrobial analyses were carried out on the hulls and edible nuts of Tetracarpidium conophorum, one of Nigerians' valuable medicinal plants belonging to the family Euphorbiaceae. Phytochemical analysis of the aqueous, ethanol and n-hexane extracts revealed the presence of alkaloids, glycosides, reduced compound sugar, carbohydrates, protein in both hull and nut. However, from proximate analysis only the hull indicated a significant amount of tannins (66%) and flavonoids (16%). Test also revealed the presence of saponins in the nut (6%). Antibacterial and antifungal evaluation of the extracts from hull and nut were done using the agar diffusion technique with serial doubling dilutions of extracts applied to appropriate wells. Statistical evaluation at 5% level of significance, indicated that there were significant differences in the responses of the tested organisms to the aqueous, ethanolic and n-hexane extracts with respect to the different concentrations; source of extract (hull or nut); pathogenic species (Staphylococcus aureus, Streptococcus pyogenes, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Aspergillus spp). However, there was no significant difference between the values obtained in relation to the state of nut(cooked and uncooked). The antimicrobial analysis revealed that the extracts from the hull had a higher antimicrobial activity. Furthermore, bacterial species were more susceptible to the extracts than fungal species. The introduction of the use of this valuable plant in the treatment of infections would be of great positive impact to the pharmaceutical industry.

TABLE OF CONTENT

Title pag	e	•				•	•	•	. i
Certifica	tion								. ii
Dedication	on								. iii
Acknow	ledgment .								. iv
Abstract									. v
Table of	Contents .								. vi
List of T	ables .								. viii
List of F	igures .								. ix
1.0 Intro	duction .	•	•	•	•	•	•	•	1
2.0 Liter	ature Review .	•	•	•	•	•	•	•	4
2.1	Origin of nuts	•				•			4
2.2	Classification of nut	s.							5
2.3	Storage and selection	n							10
2.4	Nutritional values	•	•	•		•	•		11
2.5	Medicinal value								13
2.6	Omega -3 fatty acids	s and ps	ychiatry	٧.				•	22
2.7	Tetracarpidium cond	ophorun	n and T	oxicity					24
2.8	Agricultural Importa	ince				٠	٠		27
2.9	Industrial value								28
2.10	Walnut in Pandit her	itage, r	ituals ar	nd recip	es	•			32

	2.11 Physico-chemical and functional characteristics of the conophor nut								nut	36
	2.12 Effects of	pre-sowir	ng treat	ments o	n seed g	germina	tion and	l seedlii	ng	
	growth of	Tetracai	rpidium	conop	horum					41
	2.13 Chelating ability of <i>Tetracarpidium conophorum</i>									41
	2.14 Carbohydr	ate bindir	ng spec	ificity o	of Tetra	carpidiı	ım cono	phorum	lectin	42
	2.15 Characteri	zation of	the con	ophor l	ipase	•				44
	2.16 Phytochen	nicals of 7	Tetraca	rpidium	conopi	horum			•	45
2.17 Pathogenicity of susceptible test organisms									•	46
3.0	Materials and	Methods							•	53
4.0	Results .		•	•	•	•	•	•		68
5.0	Discussion	٠							•	87
	Conclusion	•	•					•	•	90
	References	•	•					•	•	91
	Appendix					•				110

LIST OF TABLES

Гable	Title	Page					
1.	Results of Qualitative phytochemical characteristics of the						
	aqueous, ethanolic, n-hexane extracts of <i>T. conophorum</i>						
	(Hulls and Nuts)	69					
Appendix							
2.	Antimicrobial activity of ethanolic soaked hull extract	112					
3.	Antimicrobial activity of hull extract soaked in n-hexane	112					
4.	Antimicrobial activity of ethanol nut extract	112					
5.	Antimicrobial activity of ethanolic hull extract	113					
6.	Antimicrobial activity of n-hexane nut extract	113					
7.	Antimicrobial activity of n-hexane hull extract	113					
8.	Antimicrobial activity of cooked nut extract soaked in n-hexane	114					
9.	Antimicrobial activity of ethanolic cooked nut extract	114					
10.	Antimicrobial activity of hot water hull extract (dilution1)	114					
11.	Antimicrobial activity of hot water nut extract (dilution1)	115					
12.	Antimicrobial activity of cold water hull extract (dilution1)	115					
13.	Antimicrobial activity of cold water nut extract (dilution1)	115					
14.	Antimicrobial activity of hot water hull extract (dilution2)	116					
15.	Antimicrobial activity of hot water nut extract (dilution 2)	116					
16.	Antimicrobial activity of cold water hull extract (dilution 2)	116					
17.	Antimicrobial activity of cold water nut extract (dilution 2)	116					

LIST OF FIGURES

Figur	re Title	Page
.1	Photograph of the Tetracarpidium conophorum plant with fruit	8
2.	Photograph showing the allelopathic power of the <i>Tetracarpidium</i>	9
	conophorum.	
3.	a. Photograph of the raw shelled and unshelled nut of	
	Tetracarpidium conophorum	54
	b. Photograph of the cooked shelled and unshelled nut of	
	Tetracarpidium conophorum	55
4.	Graph of different concentrations of soaked Hull in Ethanol (SHE) extract	
	against zones of inhibition on tested organisms	71
5.	Graph of different concentrations of soaked Hull in n-hexane (SHN)	
	extract against zones of inhibition on tested organisms	72
6.	Graph of different concentrations of ethanol nut (EN) extract against	
	zones of inhibition on tested organisms	73
7.	Graph of different concentrations of ethanol Hull (EH) extract against	
	zones of inhibition on tested organisms	74
8	Graph of different concentrations of soxhlet n-hexane nut (NN) extract	
	against zones of inhibition on tested organisms	75
9.	Graph of different concentrations of soxhlet n-hexane Hull (NH) extract	
	against zones of inhibition on tested organisms	76
10.	Graph of different concentrations of cooked nut soaked in n-hexane	

	(CNSN) extract against zones of inhibition on tested organisms	77
11.	Graph of different concentrations of soaked cooked nut ethanol (CNE)	
	extract against zones of inhibition on tested organisms	78
12	Graph of different concentrations of hot water Hull (HWN) extract	
	(dilution 1)against zones of inhibition on tested organisms.	79
13	Graph of different concentrations of hot water nut (HWN) extract	
	(dilution1) against zones of inhibition on tested organisms	80
14	Graph of different concentrations of cold water hull (CWH) extract	
	(dilution1)against zones of inhibition on tested organisms	81
15	Graph of different concentrations of cold water nut (CWN) extract	
	(dilution1)against zones of inhibition on tested organisms	82
16	Graph of different concentrations of hot water hull (HWH) extract	
	(dilution 2)against zones of inhibition on tested organisms	83
17	Graph of different concentrations of hot water nut (HWN) extract	
	(dilution 2) against zones of inhibition on tested organisms	84
18	Graph of different concentrations of cold water hull (CWH) extract	
	(dilution 2) against zones of inhibition on tested organisms	85
19	Graph of different concentrations of cold water nut (CWN) extract	
	(dilution 2) against zones of inhibition on tested organisms	86