Phytochemical Studies on the Edible tubers of Cyperus Esculentus Linn.,

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ABSTRACT

The component acids of the oil from the tubers of *Cyperus esculentus* was investigated on silicone oil impregnated chromatographic paper. The unsaponifiable matter gave two sterols one identified as B — Sitosterol and the digitonide of the second had a melting point 218-210°C. The physical and chemical constants of the purified fixed oil were established. Total protein content of defatted meal was found to be 15.40% and the protein-hydrolysate gave fifteen amino acids. The aflatoxin content of the tubers was found to be 12.8 ug/kg.

The tubers of *Cyperus esculentus* Linn., belonging to the plant family *Cyperaceae* are commonly known as Tiger Nut (Benzon, 1957). The tubers are found abundantly in Europe, North America, Tropical Africa and Temperate zones of Asia (Manning, 1965). In Nigeria, it is found mostly in the Savannah areas in the Northern States. It has also been found to be growing well in certain parts of the Southern State (Oliver, 1960). Flour made from the tubers is a high calorie food (Chopra, 1969). Nigerians usually consume the tubers as a stimulant and appetizer, (Irvine, 1965), while in Asian countries they are used both as stimulant and as an aphrodisiac (Gray, 1950).

The tubers were collected from the Enugu market in the month of October. These were powdered in a porcelain pestle-mortar and stored in an air-tight battle.

Various authors have studied the chemical characteristics of edible seeds viz; Sesamum (Dhawan et al, 1972) and Oil Bean Tree (Kar and Okechukwu, 1978).

A systematic chemical investigation on the tubers of C. esculentus has been undertaken in the present study which essentially includes the proximate analysis of the tubers, percentage extractives in various non-polar/polar solvents, qualitative tests for the various extracts, study of the unsaponifiable fraction in the oil, physical and chemical characteristics of the fat, study of the component methyl esters of mixed fatty acids by chromatography, estimation of total proteins and study of the protein-hydrolysate by paper chromatography and finally, the determination of aflatoxin content in the defatted meal.

EXPERIMENTAL AND RESULTS

1. PROXIMATE ANALYSIS

The proximate analysis (British pharmacopoeia, 1973) of the tubers of C. esculentus were carried out and the results are stated below—

S/No.	Characteristics	Percentage w/w
1.	Moisture Content	10.66
2.	Total Ash	1.85
3.	Acid-Insoluble Ash	0.89
4.	Sulphated Ash	2.82
5.	Water-Soluble Ash	0.83

TABLE

II. PERCENTAGE EXTRACTIVES IN VARIOUS NON—POLAR/POLAR SOLVENT

500 gm of coarsely powdered tubers of C. esculentus were extracted successively in a sohlet apparatus employing solvents in an increasing order of polarity viz., Petroleum ether (40-60°C); Benzene; Chloroform; Acetone; Alcohol (95%) and Water Marc in each case was exhausted completely with the respective solvent and the later was distilled off under reduced pressure. The percentage extractives were calculated individually (see Table —I).

III. QUALITATIVE CHEMICAL EXAMINATION OF VARIOUS EXTRACTS

The qualitative chemical tests for the following plant constituents were carried out in all the extracts, viz., bitter principles and resins, alkaloids (Raffauf, 1970), carbohydrates and mucilages (Wolform and Tipson, 1968), glycosides (Trease and Evans, 1978), sterols (Klyne, 1965; Fiser and Fiser, 1959), saponins and fats and oils (Raffauf, 1970; Trease and Evans, 1978), tannins and acid compounds (Peach and Tracy, 1955); protein and amino acids (Raffauf, 1970; Wolform and Tipson, 1968; Trease and Evans, 1978).

The plant constituents, as confirmed by the above tests, are enumerated in Table I.

TABLE I

PERCENTAGE EXTRACTS AND PLANT CONSTITUENTS IN VARIOUS EXTRACTS

S/No.	Solvent Used	Percentage Extractives	Chemical Constituents
1.	Pet. Ether (40-60°C)	20.49	Oil; Sterol;
2.	Benzene	1.68	Sterol; Oil, Bitter Principle and Resin;
3.	Chloroform	2.95	Traces of alkaloid;

4.	Acetone	1.96	Carbohydrate, Glycoside;
5.	Alcohol	10.17	Carbohydrate, glycoside, Saponin; Protein and Acidic compound
6.	Water	14.37	Saponin, Tannin, glycoside, Protein and amino acid; Acidic compound.

IV. STUDY OF UNSAPONIFIABLE FRACTION

The unsaponifiable matter was separated from the purified oil by the official method (B.P. 1973). The absolute alcohol and maintained at O°C for 24 hours. The unsaponsifiable matter (0.18g) was dissolved in 10ml of absolute alcohol and maintained at 0oC for 24 hours.

A white solid residue settled at the bottom of the container which was separated by centrifugation washed with a little portion of absolute alcohol and labelled as S1.

To the alcohol soluble portion was added 10 ml of 2.5% solution of digitonin in alcohol, mixed and the contents were chilled for 24 hours. The precipitate was filtered through a fluted Whatman No. 1 filter paper, dried in air and kept in a desiccator. It was labelled as S2.

(a) Characterization of S1 and S2

Both fractions S1 (m.p. 143-145°) and S2 (m.p. 218-220°C) gave strong postive test for sterols (Klyne, 1965; Fiser and Fiser, 1959).

(b) CO-TLC of Sterol S1 and digitonide S2 with pure samples of B-Sitosterol and Stigmasterol

The TLC plates after development were sprayed with 50% Sulphuric acid in absolute ethanol (Stahl, 1969), and subsequently baked at 110°C for 10 minutes. The Rf values of various spots were calculated (see Table II)

TABLE II

R_f VALUES OF THE STEROLS

S/No.	Sterol	R_f Value	Remark
1.	Stigmasterol	0.40	One spot
2.	B–Sitosterol	0.32	One spot
3.	Sterol S_1	0.30	One spot
4.	Digitonide of Sterol S_2	0.38	One spot

V. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE OIL OF C. ESCULENTUS

The physical characteristics of the oil, e.g., specific gravity and refractive index (Deuel, 1951) and congealing temperature (I.P., 1966) and the chemical characteristics, viz., acid value, saponification value, iodine value (pyridine bromide method) and acetyl value of the fat were determined by standard method (Clowes and Coleman, 1947; B.P., 1973). All the constants are recorded in Table III.

VI. STUDY OF COMPONENT METHYL ESTERS OF MIXED FATTY ACIDS BY PAPER CHROMATOGRAPHY

Methyl esters of fatty acids resolute more distinctly than their corresponding fatty acids (De Vries, 1962; Morris, 1962). Kritchevsky and Tiselius (1951) first employed silicone oil in paper chromatography for the sepration of fatty acids and their methyl esters. The same method was adopted here besides runing a co-chromatography with five authentic samples of methyl esters of Palmitic, Lauric, Myristic, Oleic and Stearic aids.

Methyl esters of mixed fatty acids obtained from the oil of C. esculentus and pure samples of fatty acids were prepared (Shellard, 1968).

TABLE III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF OIL

S/No.	Characteristics	Observations/Values
1.	Colour	Pale Yellow
2.	Odour	Pleasant
3.	Taste	Bland and Pleasant
4.	Refractive Index (30^0C)	1.4639
5.	Weight per ml (25^0C)	0.8682 g/ml
6.	Congealing Temperature	-3^0C
7.	Acid Value	11.30
3.	Saponification Value	154.92
9.	Iodine Value	79.77
10.	Acetyl Value	84.60

Chromatographic filter papers (size 10cm x 25cm) were first washed by the ascending chromatography using a mixture of methanol, water, acetone and ether in equal proportion to remove any impurity. The treated papers were dried over fused calcium chloride in a desiccator and passed through a 2.5% v/v solution of silicone oil in petroleum ether (60-80°C) and air-dried. The silicone oil impregnated paper was spotted with the samples and allowed to develop in a pre-equlibriated chamber containing a mixture of acetic acid and water (85:15). The developed chromatogram was air-dried and the mercuric salts of the saturated methyl ester were formed by immersing it for 20 minutes in 150 ml of 0.1% mercuric acetate solution containing 0.1 ml of glacial acetic acid (A.R.). The excess of mercuric acetate was washed in running tap water for one hour. It was air-dried and subsequently sprayed with 0.2% w/v solution of diphenyl carbazide (E. Merck) in 95% ethyl alcohol and dried in the air oven at 80oC. Purple coloured spots were obtained which were stable for days. The Rf values are recorded in Table IV.

TABLE IV

R_f VALUES OF VARIOUS COMPONENT METHYL ESTERS OF FATTY ACIDS

S6No.	Methyl Esters of	No. of Spots	R_f Value	Rm Remark
1.	Mixed fatty acids	5	0.05 0.21 0.42 0.48 0.69	Separation was distinct
2.	Oleic Acid	1	0.44	
3.	Palmitic Acid	1	0.55	
4.	Lauric Acid	1	0.68	
5.	Stearic Acid	1	0.20	
6.	Myristic Acid	1	0.50	

VII. ESTIMATION OF TOTAL PROTEINS AND STUDY OF PROTEIN HYDROLYSATE BY PAPER CHROMATOGRAPHY

Total protein (15.40%) was estimated in the defatted meal by the Kjeldahl's method (Plummer, 1971). The defatted meal was hydrolysed with 50% hydrochloric acid (A.R.) and the reaction mixture was boiled for one and a half hours. It was subsequently cooled, filtered and the filtrate was treated with activated charcoal and refiltered. The volume of the final filtrate was reduced to about 15 per cent under reduced pressure.

The protein hydrolysate was found to contain fifteen amino acids out of which nine were identified with reference to pure samples as — Lysine (0.14); L-Cystine (0.20); Glutamine (0.25); L-Alanine (0.31); L-Tyrosine (0.40); L-Methionine (0.50); DL-Valine (0.61); Phenylalmine (0.64); DL-Iso-leuchine (0.68). The remaining six spots having the Rf values 0.04, 0.10, 0.76, 0.80, 0.82, and 0.85 could not be identified for want of authentic samples.

VIII. ESTIMATION OF AFLATOXIN IN THE DEFATTED MEAL OF C. ESCULENTUS

The aflatoxins present in the defatted meal were detected by standard method and estimated quantitatively (Jones, 1972).

The aflatoxin content of the tubers of C. esculentus has the average Rf value of 0.51 and the quantity estimated as 12.8 ug/kg (maximum acceptable limit in edible food being 20 ug/kg).

DISCUSSION

The tubers of C. esculentus contain negligible amount of earthy matter comparable to the seeds of X. aethiopica (Kar and Eruchalu, 1977). Low values of water-soluble ash and sulphate ash indicate the purity and low-inorganic matter of the sample. The total weight of the extractives in various non polar/polar solvents is about one half of the total weight of the tubers. They contain an appreciable quantity of fat (20.5%) comparable to those of Soyabean, Almond, Corn fritters (Watt and Merrill, 1963). The tubers contain sterol, bitter principle, and resin, traces of alkaloid, glycoside, saponin, carbohydrate, protein and amino acids.

The unsaponifiable matter (1.80%) contains two sterols, of which one is identified to be B-Sitosterol and the digitonide of the second had a melting point 218-220°C.

The physical and chemical characteristics of the purified fixed oil were established. From the chemical constants, it could be inferred that the oil of C. esculentus is fit for edible purposes. The paper chromatography of the methyl esters of the mixed fatty acids on silicone-impreganted medium revealed the presence of Oleic, Lauric, Stearic and Myristic acids. However, the exact percentage of each individual fatty acid need to be determined by VPC methods.

The total protein content in the defatted meal of the tubers of C. esculentus was found to be 15.40% which is fairly comparable to those of Wheat flour (14%); Oat flour (15.1%); Brazil Nuts (17%); Hickory nuts (15.40%) (Crosbie-Walsh, 1945). The protein hydrolysate is found to contain in all fifteen amino acids out of which five are essential amino acids viz: Lysine; DL-Valine; Phenylalamine; DL-Iso-leucine and L-Methionine besides four other amino acids, namely; L-Cystine; Glutamine; L-Alanine and L-Tyrosine and six amino acids which could not be identified. It suggests that the protein in the defatted meal has a good nutritional value.

The aflatoxin content of the tuber of C. esculentus has the Rf value of 0.51 and the quantity is found to be 12.8 ug/kg, i.e., much below the maximum acceptable value for any edible food and feeding stuff in Canada (Jones, 1972).

The tubers of C. esculentus possesses good nutritional value because of the high oil content and appreciable characteristics of the oil render it fit for human consumption. The protein is of good quality. The aflatoxin content is low and hence non-toxic to consumers.

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