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PHYTOCHEMICAL ANALYSIS AND LAXATIVE ACTIVITY OF THE LEAF EXTRACTS OF *EUPHORBIA HETEROPHYLLA* LINN (EUPHORBIACEAE)

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A hot aqueous decoction of the leaves of *Euphorbia heterophylla* Linn (Euphorbiaceae) 1.24 kg gave on cooling and defatting with dichloromethane, an aqueous solution which on successively extracting with n-butanol and ethylacetate gave 25.89g and 1.31g of residue, respectively on removal of solvent. The semi-solid extract from the ethylacetate fraction on hydrolysis with dilute tetraoxosulphate (VI) acid gave a yellow powder which on acetylation gave colourless needle clusters identified as quercetin tetracetate. The butanolic fraction had laxative action and contained saponins, phenols, terpenes and diterpenes identified as phorbols but no anthraquinones. The residual aqueous solution contained mainly sugars identified as xylose, maltose, galactose, lactose and lactulose, which are bulkforming laxatives. The purgative action was found to be a joint action of both the phorbols in the butanol fraction and the bulk forming laxative sugars in the residual aqueous fraction.

Key words**:** *Euphorbia heterophylla*, Laxative activity, Tetraoxosulphate (VI).

Introduction

Hot aqueous decoctions of *E. heterophylla* leaves have been used for ages to produce purgation in the southeastern part of Nigeria (Gill 1992). The use is so common, that it became necessary to find out scientifically the chemical principles responsible for the purgation especially as some phorbols could function as co-carcinogens (Kinsella 1987; Shaofen *et al* 1991).

In the developing countries there are about 1 billion people living in extreme poverty and vast numbers suffering and dying for want of safe water and medicine, they have no alternative for primary healthcare (WHO 1995).

The aqueous extract of the leaves of *E. heterophylla* is used by the natives to produce purgation. Infact, according to a traditional herbal practitioner when the leaves are used to cook "yam porridge" purgation ensures within 3-4 h after consumption (Sevil *et al* 1993 and 1994).

Therefore, the objectives of this study is to ascertain the claim by the users of this herb and also to investigate the chemical principle(s) responsible for the laxative activity.

Experimental

Taxonomic identification of plant material. The plant material (leaves only) was collected at the University of Benin in March 2000 and identified by Prof. L S Gill of the Department of Botany.

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Preparation and extraction. The fresh leaves were collected, weighed and washed with distilled water. 1.24 kg of the fresh leaves of *E. heterophylla* was boiled with 1.5 litres of distilled water for 10 min and filtered then evaporated at 50˚C to a syrup liquid by using a rotary evaporator. The total aqueous decoction was first extracted successively with 3 x 200 ml and 100 ml of n-butanol. The residual aqueous extract was extracted with three aliquots parts of 200 ml of ethylacetate. The butanolic fraction was defatted with 200 ml of dichloromethane. The three fractions butanol, ethylacetate and dichloromethane were evaporated to dry- ness to give 25.902g, 1.312g and 0.858g, respectively. The three fractions were subjected to phytochemical studies.

Isolation. 0.470g of the yellow semisolid obtained on removal of the solvent from the ethylacetate fraction hydrolysed with 5ml of dilute tetraoxosulphate (VI) for 30 min. The precipitate obtained (designated FH) was re-dissolved in ethylacetate and subjected to thin layer chromatographic analysis using ethylacetate : n-hexane (9:1). ^{1}H-NMR, ^{13}C-NMR and IR spectrophotometric measurements were obtained.

Acetylation. 0.197g of compound FH was acetylated with 5ml acetic anhydride and 3 drops pyridine to give 90.312 mg of colorless needle clusters (designated FA). Compound F A was subjected to ^{1}H-NMR, ^{13}C-NMR and mass spectrophotometric analysis. The melting point was also determined with the kofler melting point apparatus.

Isolation and identification of the sugars in the extracts. The cold and residual aqueous fractions after the extraction

with n-butanol and ethylacetate were gave positive test for sugars; hence, the fractions were subjected to osazone formation.

Procedure. 2.082g of the fraction were reacted with 0.5 ml phenylhydrazine, 0.5ml glacial acetic acid and 1g of sodium acetate crystals. The solution was heated in a water bath at 100°C for 30 min. The yellow osazone crystallized out about 15 min after heating. It was filtered by suction through a heated buckner funnel. The hot filtrate was again heated and crystallization occurred on cooling. The shapes of the crystals were observed under the microscope. The process was repeated until a clear solution obtained and no crystallization took place.

Due to the variations and closeness of the melting of all osazones of the common sugars, the identification of the osazones was based primarily on its crystal form (Frederick and Bernard 1960).

Chemical investigation of the butanol fraction. The three extracts including total aqueous decoction, butanol and residual aqueous fractions were screened phytochemically and the results are shown in Table 1.

The butanol fraction 1.723g was dissolved in CH_3OH : H_2O mixture (17:3) to extract any phorbol esters. The water : methanol mixture was extracted with n-hexane to remove unwanted compounds such as triterpenoids and triglyceride (Rowan and Onwukaeme 2001). The hydroalcoholic layer was further partitioned against several aliquots of diethylether, sufficient water being added to produce two layers. The ether extract containing diterpenoid esters were combined; dried over anhydrous Na_2SO_4 and evaporated to dryness to give a residue 0.035 g. The phorbol esters in the diethylether layer were detected on TLC using antimony (III) chloride and 60% H_2SO_4, with a solvent system of chloroform: diethylether (95:5). The plates were developed in the oven for 5 min at 100°C. A single greyish color spot at R_f value of 0.96 confirmed the presence of diterpene (phorbol) esters in the butanol fraction.

Table 1
Phytochemical composition of the extracts of *E. heterophylla*

Phytochemical composition	Total aqueous decoction	Butanolic fraction	Residual aqueous fraction
Carbohydrates	+	-	+
Osazone formation	+	-	+
Saponins	+	+	-
Anthranquinones	-	-	-
Alkaloids	-	-	-
Flavonoids	+	-	-
Phenols	+	+	-
Terpene	+	+	-
Diterpenes	+	+	-

+ , indicates presence of compounds; - , indicates absence of compound.

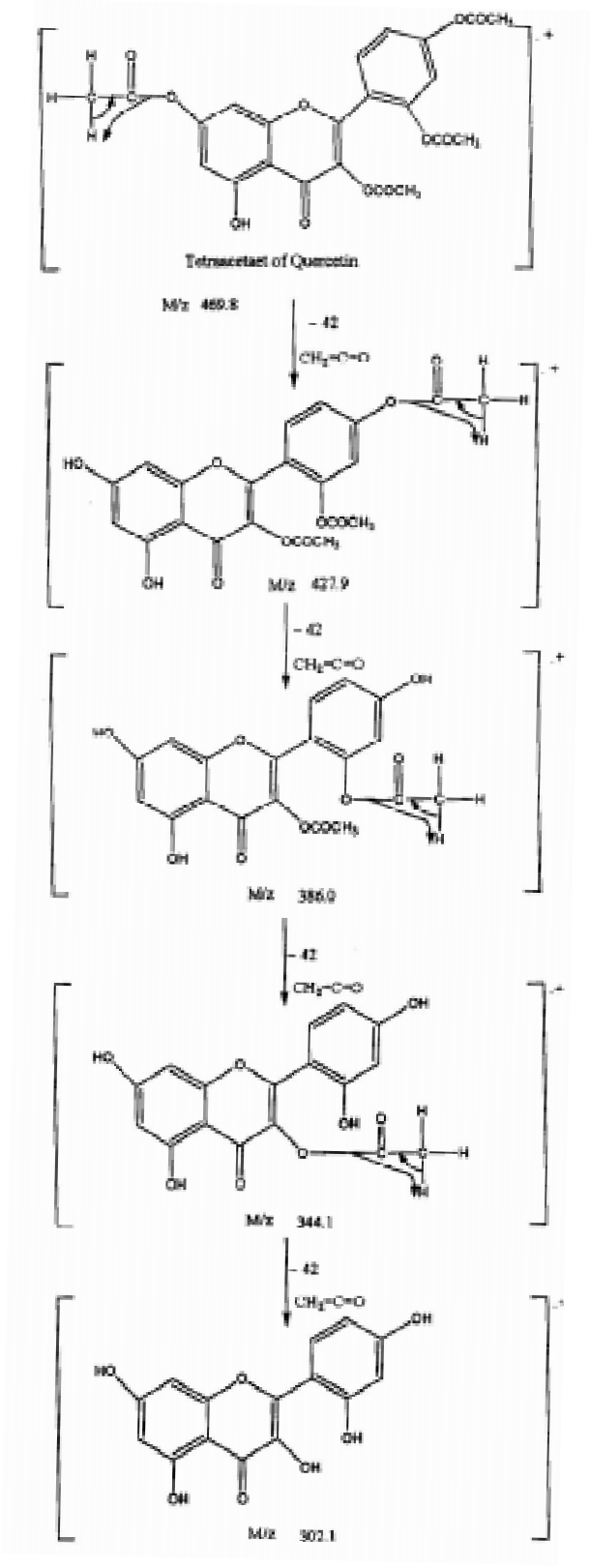
Laxative studies. The crude total aqueous decoction, butanol and the residual aqueous fractions were subjected to laxative tests using Lou's method (Lou 1949). Albino rats weighing between 115-240g were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. They were kept in well ventilated rats cages (Lou's Cages) with free access to water and feeds (ad libitum) and left in this environment for 2 weeks to acclimatize.

Administration of the extract. The rats were divided into two (2) groups of 4 each. Group 1 for the test drug (*E. heterophylla*) while group II was for the control drug (*Cassia acutifolia* leaves-herb tea). The rats were food and water starved, fasted over the night preceeding the doses and each rat was placed singly into each compartment of the cage. The next day, the faeces were examined and the rat with wet faeces was excluded from the experiment. The weights of the rats were taken.

100, 200, 300, 400, 500, 600 and 700mg/kg doses of the aqueous decoction were given to the rat in each experiment. Similarly, rats of group II were given 100mg/kg to 700mg/kg of an aqueous extract of *C. acutifolia* as control. Immediately after dosing, the rats were given the mixture of food and water mixed together (10 parts of feed plus 7 parts of distilled water) and observed at every hour for 12 h for the excretion of wet faeces which were recognized by the wire adhesion method and also by physical examination (Latven *et al* 1951). At the end of the 12 h of observation the total number of wet faeces and of dry faeces were noted. The percentage of wet faeces was also calculated.

Results and Discussion

Compound FH. Melting point, 258°C; TLC, Ethylacetate : n-hexane (9:1); ^{1}H-NMR, (400 MHZ, DMSO-d_6), δ 2.45 (DMSO-d_6), 3.25 (H_2O from DMSO), 6.15 (1H, d, H-6), 6.42 (1H, d, H-8), 7.58 (1H, d, H-6), 8.0 (1H, dd, H-3′, H-5′), 9.38 (1H, OH at H-7), 10.8 (1H, OH at H-3) 12.6 (1H, strong intramolecular H-bonded, OH at C-5 to C=O at C-4); ^{13}C-NMR, (400 MHZ, $CDCl_3$,) signals at δ 146.0 (C-2), 136.4 (C-3), 176.2 (C-4), 159.6 (C-5), 98.8 (C-6), 165.3 (C-7), 95.0 (C-8), 160.0 (C-9) 154.0 (C-10, 123.5 (C-1′), 116.8 (C-2′),



Fragmentation pattern of compound FA

Scheme 1

145.0 (C-3′) 148.6 (C-4′), 116.9 (C-5′), 123.7 (C-6′), 40.6 (DMSO); IR, (cm^{-1}), 3400 (OH, intra molecular H-bonded), 2365.8 (due to inequalities in path length), 1695 (C=O), 1168.1, 1076.9 (C-O stretching), 997.8 (C=C), 821.6 (2 isolated aromatic C-H), 880 (1 isolated aromatic C-H).

Compound FA. Acetylation of 0.1975g of compound FH gave 90.31mg of FA as colorless needle clusters.

Melting point, 165°C; TLC, Ethylacetate R_f 0.63; ^{1}H-NMR, (400 MHZ, $CDCl_3$), 1.5 (H_2O from $CDCl_3$), 7.25 ($CDCl_3$), 2.38 (9H, s, 3 acetylmethyl protons at C-4′, C-2′, C-7), 2.42 (3H, s, 1 acetylmethyl proton at C-3), 6.85 (1H, d, H-6), 7.34 (1H, d, H-8), 7.36 (1H, d, H-6′), 7.7 (1H, dd, H-3′), 7.72 (1H, dd, H-5′); ^{13}C-NMR, (400 MHZ, $CDCl_3$), 20.5 (aliphatic CH_3 of O-CO-CH_3), 77.3 (chloroform-d_6), 150.5 (C-2), 167.9 (C-3), 170.1 (C-4, C=O), 167.9 (C-7), 167.87 (C-2′), 167.8 (C-4′), 144.5 (C-5), 124.1 (C-9), 126.6 (C-10), 127.9 (C-1′); MS (70eV) M/Z, 469.8 (6%), 427.9 M^+ (64%), 386.6 (90%), 344.1 (100%), 302.1 (90%), 273.2 (20%), 245.2 (14%), 217.2 (10%); IR (cm^{-1}), 3460 (free OH), 3099.1 (-$COCH_3$), 2335 inequalities in path length), 1690 (C=O), 1168.1, 1076.9 (C-O stretching), 997.8 (C=C), 821.6 (2 isolated aromatic C-H), 880 (1 isolated aromatic C-H).

The MS for compound FA showed the presence of M^+ at 469.8 formulated as $C_{21}H_{16}O_{10}$ quercetin tetracetate requires 470.3886. The sequential loss of a four 42 units in the fragmentation pattern of FA (peaks 469.8 to 427.9 to 386.0 to 344.1 and to 302.1) indicate the loss of four ketene units arising from four acetate groups (scheme 1). The ^{1}H-NMR showed the presence of a signal at δ 2.38 integrating for 9 protons corresponding to the 9 protons of the acetyl methyl groups located at the equivalent positions C-2′, C-7 and C-4′. The 3H signal at δ 2.42 is ascribed to the 3 protons of the acetylmethyl group at (C-3).

^{13}C-NMR spectrum revealed the presence of four ester carbonyl carbons resonating at δ 167.943, 167.865, 169.370 and 170.126. The ketonic carbonyl at C-4 appeared in the spectrum at δ 170.126. This is also evident in the ^{13}C-NMR spectrum of quercetin (FH). Both FH and FA gave positive tests for flavonoids.

These data with the melting point characterised compound FA as the tetracetate of quercetin and its precursor as quercetin by inference. The phytochemical screening of the three fractions showed the absence of alkaloids and anthraquinones (known laxative agent) but showed the presence of sugars, flavonoids and diterpene (phorbol esters) (Table 1). The total aqueous decoction and residual aqueous fractions tested positive for a variety of sugars found to be xylose, lactose, glucose, galactose, maltose and lactulose. The presence of

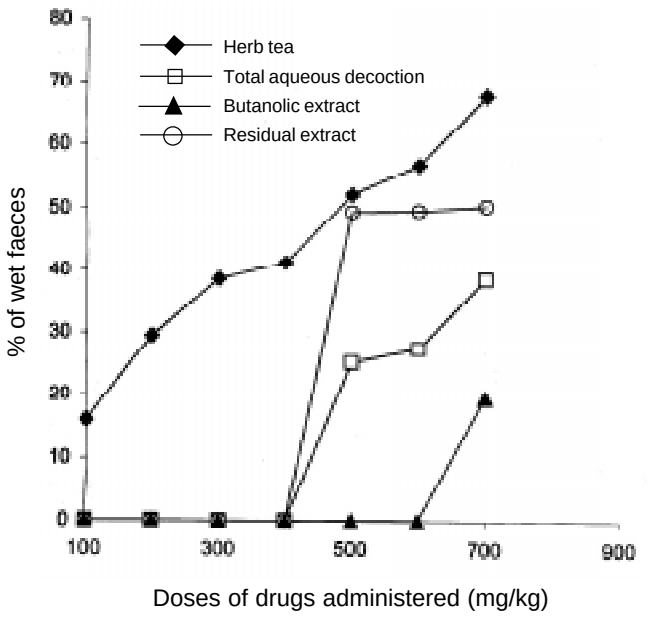


Fig 1. Graph of percentage of wet faeces produced by herb tea and fractions of *E. heterophylla.*

diterpenes (phorbol esters) was indicated in the total aqueous and butanolic fractions. The laxative studies showed a higher laxation by the residual aqueous fraction (49.3%) than that produced by the total aqueous fraction (25.2%) at doses of 500mg/kg body weight. The butanol fraction produced no laxative effect at a dose of 500 mg/kg. However, production of laxative effect at doses of 700mg/kg and above by the butanol fraction is attributable to the presence of small quantities of phorbol esters. At a dose of 700mg/kg, the laxative activity produced by the residual aqueous fraction, total aqueous decoction and the butanol fraction were 50.41%, 38.53% and 19.54%, respectively (Fig 1). Therefore, the laxative activity produced by the total aqueous decoction (38.5%) was due to the combined action of the sugars and the phorbol esters. For the butanol fraction, the 19.54% laxative effect produced at 700 mg/kg was probably due to the mild irritant action of the phorbol esters (Shaofen and Kiven 1991). On the other hand, the increased laxation produced by the residual aqueous fraction (50.41%) is due to the presence of the sugars namely xylose, glucose, lactose, lactulose and maltose, which are bulk-forming laxative by comparison with the laxative *Plantago* psyllium which is known to contain similar chemical compounds. Further studies will be carried out to isolate and characterize the phorbol esters.

Conclusion

The chemical principles responsible for the laxative activity of the leaves of *E. heterophylla* have been shown to be the sugars, xylose, lactose, galactose, glucose, lactulose and maltosc. The phorbol esters in the butanol fraction which showed complete absence of the sugars are partly responsible for the laxative activity.

Compound FH was isolated from the leaf of *E. heterophylla* and characterized as quercetin. FA (acetylated derivative of querectin) was synthesize and characterized to confirm the structure of FH.

References

Frederick G M, Bernard C S 1960 *Practical Organic Chemistry*. Longman group Ltd, London, UK, 4th ed, pp 134-145.

Gill L S 1992 *Ethanomedicinal Uses of Plants in Nigeria*. University of Benin Press, Benin City, Nigeria, p 95.

Kinsella A R 1987 A review of the evidence from *in-vitro* and *in-vivo* studies for a role for phorbol ester tumour promoters from the Euphorbiaceae in the secretion and clonal expansion of specific cell population. *Bot J Linn Soc* **94** 265-282.

Latven A R, Sloave A B, Munch J C 1951 Bioassay of carthartics emodin type. *J Am Pharm Ass Sci* **41** 548-552.

Lou T C 1949 Biological assay of vegetable purgatives. Part I. Senna leaf, fruits and their preparations. *J Pharmacol* (1), 673-682.

Rowan N G, Onwukaeme D N 2001 Diterpenoid esters of euphorbiaceae in *Euporbia hyles*. *Niger J Pharm* **32** 60-64.

Sevil O, Huching S, John M, Pezzuto, Neriman O, Geoffrey A C 1993 Biologically active compounds from Euphorbiaceae. *Plant Med* **5a** 472-474.

Sevil O, Roberto R G, Heebyung C, John M P, Geoffrey C, Ayhen U 1994 Biologically active compounds from Euphorbiaceae II. *Plant Med* **60** 594-596.

Shaofen X, Mark A C, Kiven J 1991 Phorbol esters induced oscillatory contractions of intestinal smooth muscles. *Eur J Pharm* **201**(213) 215-217.

World Health Organisation (WHO) 1995 *The World Health Reports.* Bridging the gap WHO, Geneva **1** 118.